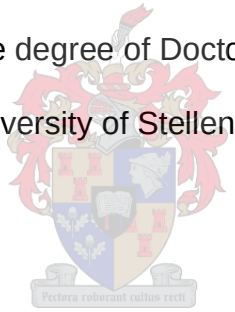
REPRODUCTION IN DWARF CHAMELEONS (*BRADYPODION*) WITH PARTICULAR REFERENCE TO *B. PUMILUM* OCCURRING IN FIRE-PRONE FYNBOS HABITAT

JENNIFER C. JACKSON

Dissertation presented for the degree of Doctor of Philosophy (Zoology) at the University of Stellenbosch



Supervisor: Prof. P le F. N. Mouton

Co-supervisor: Dr. A. F. Flemming

December 2007

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part been submitted it at any university for a degree.

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ABSTRACT

South Africa, Lesotho and Swaziland are home to an endemic group of dwarf chameleons (*Bradypodion*). They are small, viviparous, insectivorous, arboreal lizards, found in a variety of vegetation types and climatic conditions. Previous work on *Bradypodion pumilum* suggests prolonged breeding and high fecundity which is very unusual for a viviparous lizard inhabiting a Mediterranean environment. It has been suggested that the alleged prolonged reproduction observed in *B. pumilum* may be a reproductive adaptation to life in a fire-prone habitat. In addition, *Chamaesaura anguina* a viviparous, arboreal grass lizard also occurs in the fire-frequent fynbos and exhibits an aseasonal female reproductive cycle with high clutch sizes; highly unusual for the Cordylidae. With the observation of two species both inhabiting a fire-driven environment and exhibiting aseasonal reproductive cycles with high fecundity, it was thought that this unpredictable environment may shape the reproductive strategies of animals inhabiting it. However, detailed reproductive data for *B. pumilum* were unavailable. The first aim was provide baseline reproductive data for *B. pumilum* and to discuss the reproductive strategy in relation to a fire-prone environment. To establish the significance of fire in the reproductive strategy of *B. pumilum*, reproductive data of other *Bradypodion* species, not inhabiting the fire-prone area was required. The second aim was to provide baseline reproductive data for *Bradypodion* with discussion on possible scenarios facilitating the evolution of dwarf chameleon reproduction. *Bradypodion pumilum* specimens were collected in monthly samples from Stellenbosch and Somerset West in the Western Cape, South Africa. Specimens of other *Bradypodion* species were obtained from South African museums. Data were collected for both sexes of *Bradypodion*, and sperm storage

ability was investigated in *B. pumilum*. *Bradypodion* females all showed an aseasonal reproductive cycle with relatively high clutch sizes for their body size and the possibility of individual females producing multiple clutches per year. Male *Bradypodion* have sperm available the entire year round however, there appears to be an increase in sperm production in autumn and again in spring in *B. pumilum*. Elements of this bimodal pattern are seen in other *Bradypodion* species. Dwarf chameleons regardless of habitat and associated climatic conditions are thus able to reproduce through out the year. It has also been demonstrated in *B. pumilum* that both sexes are able to store sperm and it is expected that other *Bradypodion* species would posses this character. This type of reproductive strategy is highly unusual for viviparous, temperate-zone lizards. It is likely that the cooling of the climate due to the development of the Benguela current facilitated the transition to viviparity in *Bradypodion*. *Bradypodion* may be aseasonal reproducers for a number of reasons. They are of tropical ancestry, they relatively recently inhabited tropical forests, or fluctuations in climate may have caused this. *Bradypodion* most likely have a high reproductive output due to their intense vulnerability to predation as in other chameleon species. The proposed hypothesis that the unusual reproductive characteristics of *B. pumilum* (and possibly the ancestral *Bradypodion*) were due to inhabiting a fire-prone environment now appears an unlikely explanation. However, even if this extraordinary reproduction was not in direct response to fire, the strategy appears beneficial in this type of unpredictable environment.

UITTREKSEL

Suid-Afrika, Lesotho en Swaziland huisves ‘n endemiese groep van dwergverkleurmannetjies (*Bradypodion*). Hulle is klein, lewendbarende, insekvretende, arboreale akkedisse en word gevind in ‘n verskeidenheid van plantegroeitipes en klimaatsomstandighede. Vorige studies op *Bradypodion pumilum* dui op ‘n verlengde broeiseisoen en hoë fekunditeit, wat ongewoon is vir ‘n lewendbarende akkedis wat in ‘n Mediterreense omgewing voorkom. Daar is voorheen voorgestel dat die skynbare verlengde voortplanting in *B. pumilum* ‘n aanpassing tot oorlewing in hoogs brandvatbare habitat kan wees. *Chamaesaura anguina* is ook ‘n lewenbarende, arboreale akkedis wat in fynbos voorkom wat hoogs vatbaar is vir brande en groot werpsels produseer en ‘n aseisoenale voortplantingsiklus in wyfies toon; hierdie patroon is ongewoon vir lede van die Cordylidae. Met die waarneming dat twee species wat in ‘n brandvatbare omgewing voorkom albei aseisoenale voortplantingsiklusse en hoë fekunditeit toon, het die gedagte ontstaan dat hierdie onvoorspelbare omgewing die voortplantingstrategieë van diere wat daarin voorkom, bepaal. Gedetailleerde voortplantingsdata ontbreek egter vir *B. pumilum*. Die eerste doelstelling van die studie was dus om basisinligting te voorsien oor voortplanting by *B. pumilum* en om die voortplantingstrategie aan die hand van die brandvatbare omgewing te bespreek. Om die moontlike rol van brand in die vorming van die voortplantingstrategie van *B. pumilum* te ondersoek, is voortplantingsdata vir ander *Bradypodion* species wat nie in brandvatbare habitat voorkom nie, nodig. Die tweede doelstelling was dus om basisinligting oor voortplanting by *Bradypodion* in die breë in te samel, gevolg deur bespreking van moontlike scenarios in die evolusie van voortplanting by dwergverkleurmannetjies.

Bradypodion pumilum eksemplare is maandeliks versamel te Stellenbosch en Somerset-wes in die Weskaap, Suid-Afrika. Eksemplare van ander *Bradypodion* species is vanaf Suid-Afrikaanse museums verky. Data is vir beide geslagte van *Bradypodion* versamel, en die vermoë tot spermstoring in *B. pumilum* bepaal. *Bradypodion* wyfies het almal ‘n aseisonale voortplantingsiklus getoon met relatief hoë werpselgroottes vir hul liggaamsgrootte en daar bestaan die moontlikheid dat individuele wyfies verskeie werpsels per jaar kan lewer. *Bradypodion* mannetjies produseer sperms dwarsdeur die hele jaar, maar daar blyk tog ‘n toename in spermstoring te wees in die herfs en weer in die lente in *B. pumilum*. Spore van hierdie bimodale patroon word in ander *Bradypodion* species gesien. Dwergverkleurmannetjies is dus instaat om dwardeur die jaar voort te plant, ongeag die habitat en geassosieerde klimaatsomstandighede. Daar is getoon dat beide geslagte van *B. pumilum* sperms kan stoor en daar word verwag dat ander *Bradypodion* species ook hierdie vermoë het. Hierdie tipe van voortplantingstrategie is ongewoon vir lewendbarende akkedisse van die gematigde sone. Dit is moontlik dat die ontwikkeling van ‘n koue klimaat weens die onstaan van die Benguela-stroom aanleiding gegee het tot die oorskakeling na lewendbarendheid in *Bradypodion*. *Bradypodion* mag aseisonale voortplanting toon vir ‘n aantal moontlike redes. Hulle is van tropiese oorsprong, het redelik onlangs tropiese woude betrek, of fluktuasies in klimaat kon ook die oorsaak wees. *Bradypodion* het waarskynlik hoë voortplantingsuitset omdat hulle besonder kwesbaar is vir predasie, soos dit die geval is by ander verkleurmannetjies. Die aanvanklike hipotese dat die ongewone voortplantingseienskappe van *B. pumilum* (en moontlik die voorvaderlike *Bradypodion*) ‘n gevolg is van lewe in ‘n hoogs brandvatbare omgewing, blyk nou ‘n onwaarskynlik te wees. Selfs as hierdie

buitengewone voortplantingstrategie nie ‘n direkte gevolg van brandvatbaarheid is nie, blyk die strategie voordelig te wees vir oorlewing in hierdie onvoorspelbare omgewing.

ACKNOWLEDGEMENTS

Supervisors/Reviewers: Thanks to le Fras and Alex for constant support, suggestions and even the occasional argument! To Darren Houniet, John Measey, Krystal Tolley and Sandi Willows-Munro for proof-reading this thesis and making valuable suggestions – Thank you.

Permits/Funding: Thanks to CapeNature for the collection permit, and the Critical Ecosystem Partnership Fund and the National Research Foundation for funding.

Chameleon hunters: Thanks to Briggitte Bruyns, Susana Clusella-Trullas, Eloise Costandius, Jacques Deere, Dahné du Toit, Lawrence Dunn, Rebecca Fell, Kev Hopkins, Sam Hopkins, Darren Houniet, Pete le Roux, Cindy Shuttleworth, Kelvin Shuttleworth, Krystal Tolley, Sandi Willows-Munro and last but certainly not least, Tomas Lado who found the most chameleons with the smallest torch. Without your help there would be no project.

Museums: Thanks to Bill at the Port Elizabeth Museum (Bayworld), Lemmy at the Traansvaal Museum and Debbie at the Natal Museum for your hospitality, use of your collections and for providing me with ample work space during my visits.

Emotional Support: Being the worry-bug that I am this thesis would not be finished without these special people. My family and friends in England, my flat mates Sam Hopkins, Jacques Deere, Kevin Hopkins, Sara Haveron, my wonderful partner Darren Houniet, and finally a special thanks to my Mummybear for her amazing ability to make me feel better regardless of the situation.

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CHAPTER ONE

AN INTRODUCTION TO SOUTHERN AFRICAN LIZARD REPRODUCTION WITH PARTICULAR REFERENCE TO THE DWARF CHAMELEONS

SQUAMATE REPRODUCTION

The evolution of viviparity

Oviparous squamates (lizards, snakes and amphisbaenians) generally lay eggs after considerable in utero embryonic development is completed (Blackburn 1995). Viviparous squamates, on the other hand, retain eggs in the maternal reproductive tract until embryonic development is complete (Blackburn 1995). Many squamates are viviparous and viviparity has evolved more than 100 times in them (Blackburn 1999; 2000). They are usually late maturing seasonal reproducers; most having a single clutch of large young per year (Tinkle 1969; Dunham *et al*. 1988).

There are many hypotheses concerning the evolution of viviparity in squamates but the most commonly accepted is the 'cold climate' hypothesis. This states (assuming that increased embryonic development at lower temperatures can not be selected for) that viviparity arose from oviparity by females retaining eggs internally, in cold climates to provide an enhanced thermal environment for developing embryos (Shine 1985; 1995). The high numbers of viviparous reptiles found at high altitudes and high latitudes (associated with cold climates) support the 'cold climate' hypothesis (Blackburn 1982; Shine 1985).

In cold climates, the mother provides a better thermal environment for developing embryos than the soil and retained eggs, even if retained for only short periods, can develop faster in cold climates than eggs in nests (Andrews 2000). The enhanced thermal environment provided by the mother as well as increasing the embryonic developmental rate may also enhance offspring traits associated with fitness (Shine 1985a; Van Damme *et al.* 1992; Ji and Brana 1999; Deeming 2004).

In addition, the risk of mortality from temperature or humidity extremes in the nest will also be avoided. However, viviparity is generally considered more costly than oviparity as gravid females may be less able to escape from predators due to the physical constraints of being pregnant (Shine 1980; Cooper *et al*. 1990).

The proliferation of viviparity

Although squamate viviparity originated in cold environments there are numerous viviparous species occurring in warm temperate and even tropical areas (Fitch 1970; Licht 1984). It is thought that thermal manipulation of the embryonic environment by the mother, which enabled viviparity to evolve, is also responsible for the radiation of viviparous reptiles into tropical areas (Webb *et al.* 2006). Careful manipulation of the mother's body temperature may have a beneficial effect (i.e. increase offspring fitness and hence survival) in both temperate and tropical areas (Andrews 2000; Shine 2004a; 2004b; Webb *et al.* 2006).

Reptile reproductive cycles

Reptilian reproduction can be affected by phylogeny, sex, habitat, climatic conditions and geographical distribution but in general two basic reproductive cycles are known, aseasonal and seasonal (Licht 1984). In temperate regions, reptiles generally show seasonal breeding whilst tropical reptiles may show either seasonal or aseasonal breeding, subject to resource availability and environmental conditions. Seasonal breeding reptiles have set periods of reproductive activity and inactivity whereas aseasonal breeders (at the population level) can reproduce throughout the year. Due to the climatic seasonality in temperate regions, conditions such as temperature, precipitation and photoperiod may initiate different

stages of the reproductive cycle of reptiles. This seasonality may have indirect affects on other resources such as food availability and hence reproduction. Reptile reproductive aseasonality is typically found in tropical areas and although uncommon, is also seen in temperate areas (Du Toit *et al.* 2003). Aseasonal reproductive cycles may be associated with areas of extreme unpredictability (Du Toit *et al.* 2003). Licht (1984) defines reproductive aseasonality as all stages of the reproductive cycle observed in equal numbers throughout the year. However, many authors refer to aseasonality when reproductive activities occur throughout the year. Aseasonality as defined in Licht (1984) is rare in both temperate and tropical environments.

Reptilian sperm storage

Male and female reptiles can store sperm, the males in their epididymis and vas deferens and the females in their oviduct. Seasonally reproducing males may store sperm if maximum sperm production occurs after the mating season, retaining sperm until the following mating season. Many reasons have been suggested for the evolution of female sperm storage, and include, the fertilisation of further clutches (Conner and Crews 1980), to synchronise male and female reproductive cycles (Cohen 1977), for cryptic female choice (Eberhard 1998) and, to minimise copulation frequency (Conner and Crews 1980; Birkhead and Møller 1993).

Within the female reproductive tract specialised structures for the storage of sperm (sperm storage tubules) have been found in many members of lizard families; in the infundibular region in members of the Agamidae, Anguidae, Eublepharidae,

Gekkonidae, Iguanidae, Scincidae and in the vaginal region in the Agamidae, Chamaeleonidae, Polychrotidae and Iguanidae (Sever and Hamlet 2002, and references therein). It is thought that these tubules help to maintain the sperm for prolonged periods although specialised structures may also be used for short term sperm storage (Flemming 2006).

LIZARD REPRODUCTION IN SOUTHERN AFRICA

Southern Africa has a diverse lizard fauna with more than 300 species described to date, many of which show a high degree of endemism (Branch 1998; Bauer 1999). Eight lizard families are represented in the southern African region; they are Scincidae, Lacertidae, Gerrhosauridae, Cordylidae, Varanidae, Chamaeleonidae, Gekkonidae and Agamidae.

Of the eight southern African families at least one species of each family, with the exception of Gerrhosauridae, is represented by a detailed reproduction study (Table 1.1). The majority of studies are on the Cordylidae with nine species represented in the literature, although all families appear grossly underrepresented in comparison to the total lizard diversity of southern Africa. Of the 26 species represented there are 17 oviparous and 9 viviparous species and, eight are from tropical areas and 18 are from temperate areas. There are several reproductive characters that are notably atypical within the southern African region; aseasonal reproductive cycles in temperate areas, viviparous species with aseasonal reproductive cycles and r-selected viviparous lizards in temperate areas. The full extent of the variation in lizard reproduction in southern Africa is largely unknown due to the dearth of scientific studies. Southern African lizards also show more

variability in reproductive cycling than northern hemisphere species (Fitch 1970; Licht 1984; James and Shine 1985). In addition, the reproductive strategies of Australian lizards occurring in environments somewhat comparable to southern Africa (temperate environments) appear more conservative than southern African species, with reproduction confined to spring and summer (James and Shine 1985). Conversely, tropical Australian lizards may breed continuously or in either the wet or dry season as in southern Africa (James and Shine 1985a; Shine 1985b).

Dwarf chameleons (Bradypodion)

Dwarf chameleons are small, viviparous, insectivorous, arboreal lizards distributed in southern Africa (Figure 1.1) in a variety of vegetation types and climatic conditions (Figure 1.2). Two separate origins of viviparity occurred in the Chamaeleonidae and *Bradypodion* represents one of them (Blackburn 1999). There are currently 15 recognised *Bradypodion* species (Branch 1998; Branch *et al*. 2006) with the possibility of a number of new distinct lineages that need further investigation (Tolley *et al.* 2004; Tolley and Burger 2007).

Previous reproductive studies on Bradypodion

Langewerf (1992) made anecdotal observations on the reproductive cycle of *B. thamnobates* in a study based on a relocated, captive population in America but reproductive cycles of captive lizards may be abnormal (Blackburn *et al*. 2003). Burrage (1973) studied the ecology of *B. pumilum* and proposed a prolonged breeding season; an unusual strategy for a viviparous, temperate-zone lizard. However, he sampled widely and included chameleons that are now known to be

species other than *B. pumilum*. Consistent frequent sampling, with large sample sizes, is therefore required for accurate interpretation of a prolonged female reproductive cycle. The male cycle of *B. pumilum* is also unclear as Burrage (1973) states only that testes are inactive in June and July. Veith (1974) in a study on the maintenance of pregnancy showed that *Bradypodion pumilum* stores sperm, however upon inspection of his photomicrographs the sperm storage receptacles did not appear to contain sperm and may in fact just be folds in the vagina. Atsatt (1953) in a short note on captive reproduction in *B. pumilum* proposed sperm storage because a female became pregnant without mating. Busack and Busack (1967) also wrote a short note on growth rates of *B. pumilum* and showed multiple clutching in *B. pumilum*, but only had one captive chameleon.

Table 1.1 The reproductive particulars for southern African lizard species studied in detail. V = viviparity, O = oviparity, M = reproductive mode, RA = reproductive activity, CS = clutch size, C/Yr = clutches per year, asterisk indicates Namaqualand population only, double asterisks indicates some specimens were captive.

SPECIES	M	FEMALE RA	MALE RA	CS	C/Yr	CLIMATE	REFERENCE
Trachylepis capensis	V	Winter-spring	Pre-nuptial	8 – 20	1	Temperate	Flemming 1994
Trachylepis quinquetaeniata	O	Spring-summer	Aseasonal	2 – 9 (4.8)	>1	Tropical	Simbotwe 1980
Trachylepis striata	V	Aseasonal	Aseasonal	2 – 20 4.2)	>1	Tropical	Simbotwe 1980
Meroles anchietae	O	Aseasonal	Aseasonal	1 – 2 (1.3)	2 – 4	Subtropical	Goldberg & Robinson 1979
Meroles cuneirostris	O	Spring-summer	Post-nuptial	1 – 4 (2.9)	2	Subtropical	Goldberg & Robinson 1979
Varanus albigularis	O	Autumn-winter	Unknown	8 – 51	1	Subtropical	Phillips & Millar 1998; Branch 1998
Chamaesaura anguina	V	Aseasonal	Post-nuptial	3 – 17 6.4)		Temperate	Du Toit *et al.* 2003
Cordylus cataphractus	V	Autumn-winter	Pre-nuptial	1	1	Temperate	Flemming & Mouton 2002
Cordylus giganteus	V	Autumn-winter	Post-nuptial	2	<1	Temperate	Van Wyk 1991; 1995
Cordylus polyzonous	V	Autumn-winter	Pre-nuptial	1 – 5 (2.85)	1	Temperate	Flemming & Van Wyk 1992; Van Wyk 1990; Flemming 1993a
Platysaurus capensis	O	Autumn-winter	Pre-nuptial	2	>1	Temperate	Broadley 1974; Van Wyk & Mouton 1996
Platysaurus minor	O	Autumn-winter	Pre-nuptial	2	>1	Temperate	Broadley 1974; Van Wyk & Mouton 1996

Table 1.1 continued The reproductive particulars for southern African lizard species studied in detail. V = viviparity, O = oviparity, M = reproductive mode, RA = reproductive activity, CS = clutch size, C/Yr = clutches per year, asterisk indicates Namaqualand population only, double asterisks indicates some specimens were captive.

SPECIES	M	FEMALE RA	MALE RA	CS	C/Yr	CLIMATE	REFERENCE
Pseudocordylus microlepidotus	V	Autumn-winter	Post-nuptial	3 – 7 (4)	1	Temperate	Within Van Wyk & Mouton 1998; Branch 1998
Pseudocordylus capensis	V	Autumn-winter	Pre-nuptial	2	1	Temperate	Van Wyk & Mouton 1998
Pseudocordylus melanotus	V	Autumn-winter	Post-nuptial	1 – 6 (3.45)	1	Temperate	Flemming 1993b; 1993c
Agama aculeata	O	Spring-summer	Unknown	8 – 17 (11.5)	2	Subtropical	Heideman 1994; Branch 1998
Agama atra	O	Spring-summer	Pre-nuptial	8 – 14	2	Temperate	Van Wyk 1983; 1984a; 1984b; Van Wyk & Ruddock 2002
Agama atra atra *	O	Aseasonal	Aseasonal			Temperate	Mouton & Herselman 1994
Agama planiceps	O	Spring-summer	Unknown	4 – 9 (6.0)	1	Subtropical	Heideman 1994; Branch 1998
Acanthocerus a. atricollis	O	Spring-summer	Spring-summer	11.36	1	Temperate	Reaney & Whiting 2002
Chamaeleo namaquensis	O	Autumn-spring	Aseasonal	6 – 22 (13.2)	2 – 3	Temperate	Burrage 1973
Chondrodactylus angulifer	O	Spring-summer	Unknown	1.92	>1	Temperate	Pianka & Huey 1978; Branch 1998
Colopus wahlbergii	O	Summer	Unknown	2	U	Subtropical	Pianka & Huey 1978; Branch 1998
Pachydactylus capensis	O	Spring-summer	Unknown	1 – 2	>1	Temperate	Bates 1991
Pachydactylus bibroni	O	Winter-spring	Pre-nuptial	2	>2	Temperate	Flemming & Bates 1995
*Pachydactylus laevigatus***	O	Winter-spring	Unknown	2	3	Temperate	Werner 1977
Ptenopus garrulus	O	Spring-summer	Spring-summer	1	2	Temperate	Hibbits *et al.* 2005

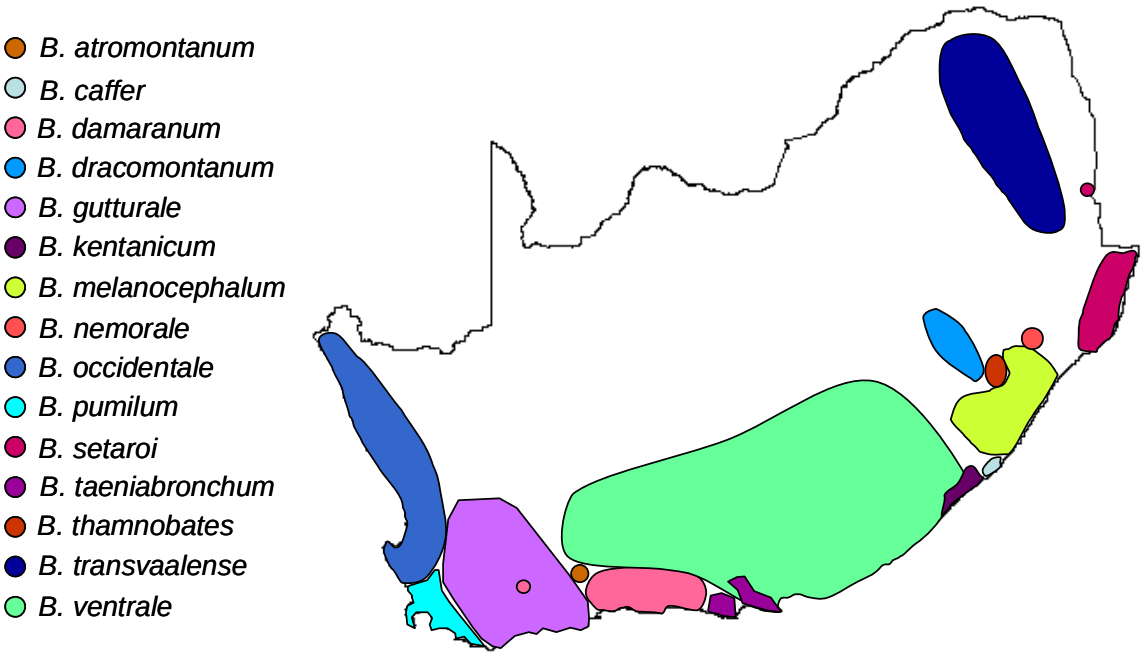


Figure 1.1 Outline of Southern Africa showing the approximate distributions of the *Bradypodion* species (Branch 1998; Tolley and Burger 2007).

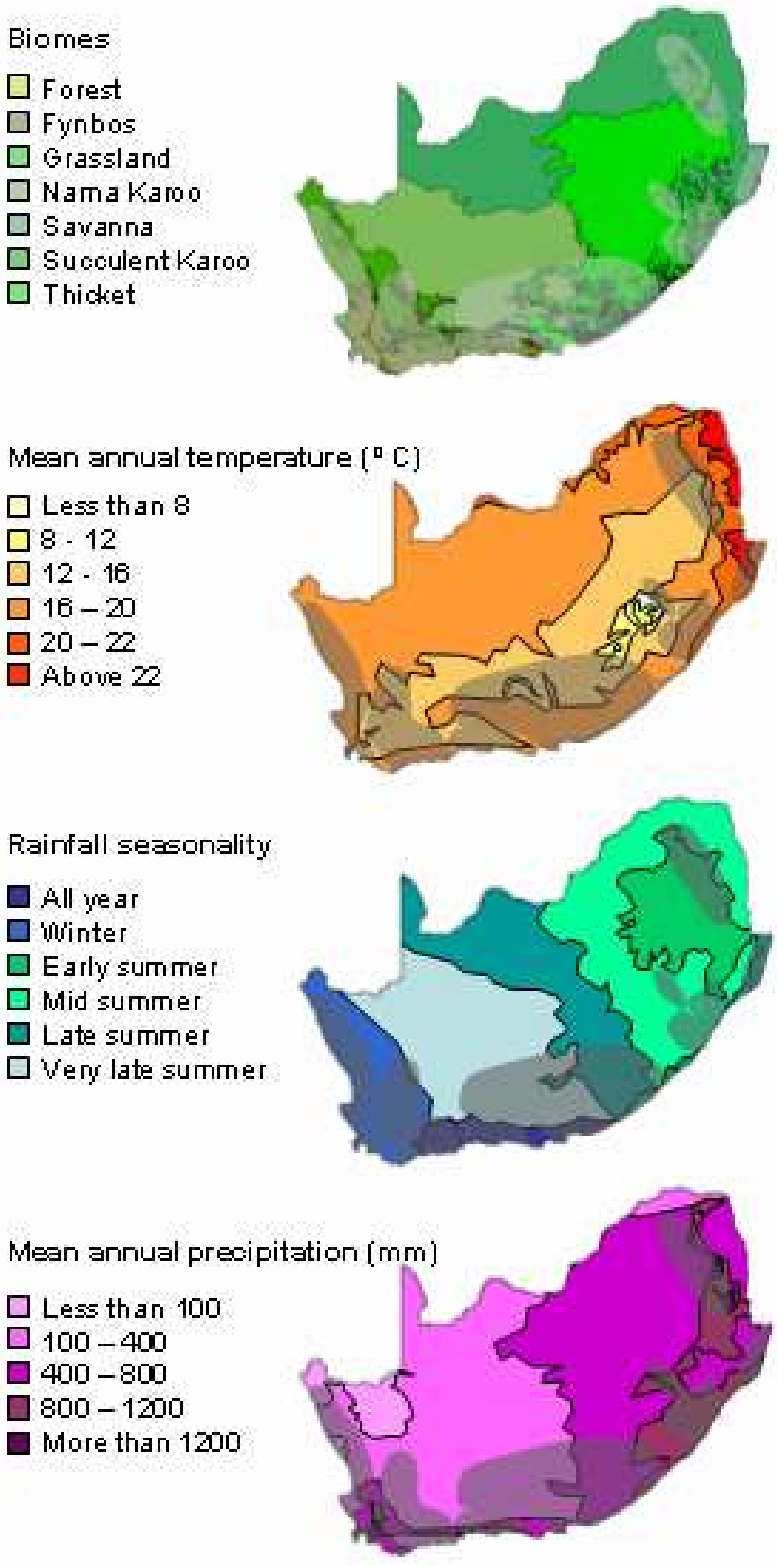


Figure 1.2 Maps showing the different biomes and climatic conditions in South Africa, Lesotho and Swaziland with *Bradypodion* species distributions overlaid in grey. Maps are modified from Low and Rebelo (1998) and Schulze (1997).

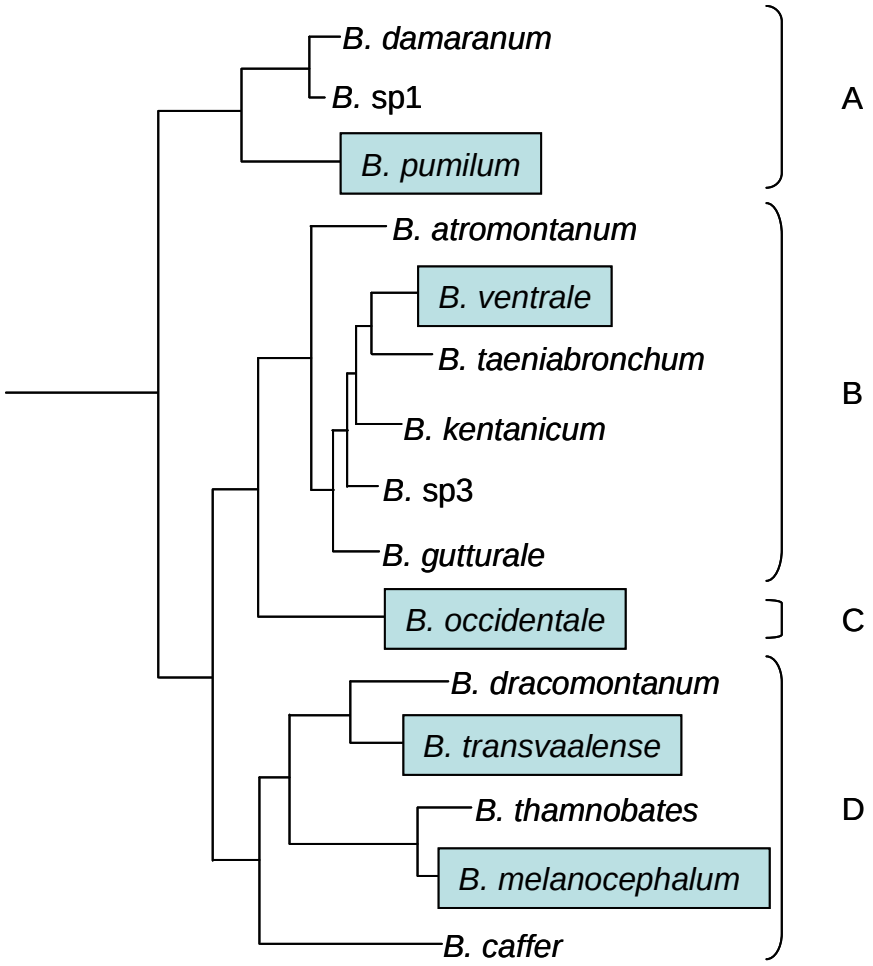


Figure 1.3 Modified phylogeny of the dwarf chameleons, in the genus *Bradypodion* (from Tolley *et al.* 2006; Branch *et al.* 2006a). Clades (A - D) are well supported. Shaded blocks show species selected for this study.

MOTIVATION AND AIM

Previous work suggests that *B. pumilum* exhibits an unusual reproductive strategy for a viviparous lizard with prolonged breeding and high fecundity in a Mediterranean environment (Burrage 1973). *Bradypodion pumilum* is found in a wide range of vegetation types, including renosterveld, strandveld, fynbos, and it is even successful in urban gardens and parks (Branch 1998; Tolley and Burger 2007). Fynbos is a shrubland endemic to south-western Africa that is susceptible to burning and indeed needs fires to thrive (Cowling and Richardson 1995). Given that other species inhabiting fire-driven areas are known to have adaptations for survival in this environment, it was suggested that the alleged prolonged reproduction observed in *B. pumilum* may be a reproductive adaptation to an unpredictable environment (Du Toit *et al.* 2003). In addition, *Chamaesaura anguina* a viviparous, arboreal grass lizard also occurring in fynbos exhibits an aseasonal female reproductive cycle with high clutch sizes; highly unusual for the Cordylidae (Du Toit *et al.* 2003). *Chamaesaura anguina* is known to be a fire-sensitive species with high direct mortality and low post-fire survival (Du Toit 2001). It is suggested that the unusual reproductive strategy observed in *C. anguina* is advantageous in an unpredictable environment as aseasonal reproduction would help to ensure that the entire yearly reproductive effort is not lost in a single fire. Large clutches in this animal would assist in rapid repopulation of the area after a fire. With the observation of two species both inhabiting a fire-driven environment and exhibiting aseasonal reproductive cycles with high fecundity, it was thought that this unpredictable environment may shape the reproductive strategies of animals inhabiting it (Du Toit *et al.* 2003). The observation of a prolonged breeding season in *B. pumilum* therefore begs for

further investigation. However, the alleged unusual reproductive strategy of *B. pumilum*, although it may appear beneficial in a fire prone habitat, may not be an adaptation to fire itself. Studying species related to *B. pumilum* occurring in other habitat types may provide insights into the adaptive nature of alleged prolonged breeding in this species.

The aim of the present study is twofold. First is to conduct a detailed investigation into the reproductive parameters of the dwarf chameleons (*Bradypodion*), with particular reference to the Cape Dwarf Chameleon, *Bradypodion pumilum*. The reproductive cycle of *B. pumilum* is revisited due to the inadequacies of Burrage's (1973) study. Large sample sizes and frequent sampling from a small geographic area are necessary to accurately interpret the reproductive strategy of *B. pumilum*. Second is to discuss possible scenarios in the evolution of *Bradypodion* reproductive strategies, specifically looking at the possible effects of fire in *B. pumilum*.

As a representation of reproductive strategies in all *Bradypodion* species, *B. melanocephalum, B. occidentale, B. pumilum, B. transvaalense* and *B. ventrale* were examined in detail. These five species are representative of all of the well supported clades in the *Bradypodion* phylogeny (Tolley *et al.* 2004; Tolley *et al*. 2006; Figure 1.3) and included taxa occurring in different vegetation types and climate regimes of southern Africa (Figure 1.2).

I hypothesise that as the dwarf chameleons are viviparous and from temperate southern Africa they should reproduce seasonally and follow a K-selected

strategy, unless they inhabit an unpredictable environment. For that reason, I predict that *B. pumilum* would show a reproductive adaptation to inhabiting fynbos. However, if other members of the *Bradypodion* (not inhabiting fire-prone areas) show a similar reproductive strategy to the suggested prolonged breeding of *B. pumilum*, then inhabiting a fire-prone environment may not be the main driver for this reproductive strategy.

CHAPTER TWO

REPRODUCTIVE ASYNCHRONY IN FEMALE CAPE DWARF CHAMELEONS (*BRADYPODION PUMILUM*) IN A MEDITERRANEAN ENVIRONMENT

INTRODUCTION

Viviparity

There are many hypotheses concerning the evolution of viviparity in squamates but the most commonly accepted is the 'cold climate' hypothesis. This hypothesis states that viviparity arose from oviparity by females retaining their eggs in cold climates, associated with high altitudes and high latitudes, to increase the fitness of their offspring (Shine 1985a). Although squamate viviparity originated in cold environments there are numerous viviparous species occurring in warm temperate and even tropical areas (Fitch 1970; Licht 1984). It is thought that thermal manipulation of the embryonic environment by the mother, which enabled viviparity to evolve, is also responsible for the radiation of viviparous reptiles into tropical areas (Webb *et al.* 2006). Careful manipulation of the mother's body temperature may have a beneficial effect (i.e. increase offspring fitness and hence survival) in both temperate and tropical areas (Andrews 2000; Shine 2004a; 2004b; Webb *et al.* 2006). Viviparous lizard species usually mature late and produce a single clutch of few large young per year (Tinkle 1969; Dunham *et al*. 1988). Chameleons may be exceptional in this regard, as anecdotal information suggests viviparous forms are highly r-selected (Dunham *et al*. 1988).

Reproductive cycles

In addition to the mode of reproduction, reptilian reproductive cycles can be affected by many things such as; phylogeny, sex, habitat, climatic conditions and geographical distribution (Licht 1984). Two general reproductive patterns are recognised in reptiles; aseasonal and seasonal breeding. In temperate regions, seasonal variation in climatic conditions such as temperature, precipitation and

photoperiod may initiate different stages of the reproductive cycle of reptiles. The seasonality of biotic factors, such as food availability, may influence the accumulation of fat body reserves thereby indirectly affecting the reproductive cycle. For seasonally reproducing females, Licht (1984) suggests pre-nuptial and post-nuptial vitellogenic cycles. Pre-nuptial vitellogenesis, most commonly observed in lizards and snakes, is where the individual is pre-vitellogenic for most of the year and then rapid ovarian growth occurs just before spring ovulation. In post-nuptial vitellogenesis, follicles begin to grow in mid to late summer and continue steady growth until ovulation the following spring. Aseasonal breeding, although uncommon, may also be found in temperate reptiles (Du Toit *et al.* 2003). Tropical reptiles may show either seasonal or aseasonal breeding, depending on resource availability and environmental conditions (Licht 1984). Reptiles inhabiting sub-tropical regions with distinct seasonal periods are generally seasonal reproducers whilst reptiles inhabiting the aseasonal tropics may reproduce continuously. Even within the tropics, very few reptiles are considered truly aseasonal. True aseasonality would be represented by equal numbers of individuals, in each reproductive stage, present throughout the year (Licht 1984).

The Cape Dwarf Chameleon, **Bradypodion pumilum**

The Cape Dwarf Chameleon, *Bradypodion pumilum*, is a small, arboreal, insectivorous, viviparous dwarf chameleon from the Western Cape, South Africa (Branch 1998; Tolley and Burger 2007). The Western Cape has a Mediterranean climate (Schulze 1997; Midgley *et al.* 2005). *Bradypodion pumilum* inhabits a variety of vegetation types, including renosterveld, strandveld, fynbos and it is even successful in urban gardens. Burrage (1973) implied a prolonged breeding

season, high clutch sizes and multiple clutching for *B. pumilum*; generally considered an unusual strategy for viviparous, temperate-zone lizards. However, consistent frequent sampling, with large sample sizes, is required for an accurate interpretation of a prolonged reproductive cycle. In addition, specimens collected in Burrage (1973) were from localities now known to be within the range of other dwarf chameleon species.

AIM

Given that viviparous reptile species inhabiting temperate regions are generally K-selected, the previously reported reproductive characteristics of *B. pumilum* are highly intriguing. Burrage's (1973) observations for this species may be compromised due to sampling errors, but if indeed true, may be of particular importance as this species occurs in fire-driven vegetation. At least one other species, *Chamaesaura anguina*, shows this type of reproductive strategy in fynbos (Du Toit *et al.* 2003). Therefore my aim was to produce a detailed description of the female reproductive parameters of *B. pumilum*, focussing specifically on fecundity, seasonality and synchronicity amongst individual females. The results will be discussed in relation to *B. pumilum* inhabiting a fire-driven environment.

In view that oviparous chameleons are typically early maturing animals with high fecundity (Dunham *et al.* 1998), I hypothesise that *B. pumilum* would follow a similar strategy despite being viviparous. This type of reproductive strategy would seem particularly advantageous in a fire-driven environment.

MATERIALS AND METHODS

Study site and collection of female specimens

As mentioned previously, the Western Cape of South Africa has a Mediterranean climate characterised by high winter rainfall and a dry summer period (Schulze 1997; Midgley *et al.* 2005). Average monthly temperatures in º C (weather stations Strand: 0005609 8 and Paarl: 0021823 0), and monthly rainfall in mm (weather stations Strand: 0005609 8, Paarl: 0021823 0, Stellenbosch: 0021656A6 and Somerset West: 0005603 7) were obtained from weather stations close to the study area (from WeatherSA.com), from February 2005 until January 2006. The weather station data were used to calculate overall monthly means of temperature and rainfall for the study period (Figure 2.1).

Female *Bradypodion pumilum* specimens were collected in monthly samples from Stellenbosch (3318DD) and Somerset West (3418BB), between February 2005 and January 2006 (Figure 2.2). Stellenbosch and Somerset West were chosen as sites because of the semi-natural, relatively stable vegetation found there. The lizards were sacrificed within 24 hours of capture, fixed in 10 % formalin and preserved in 70 % ethanol. The specimens were deposited in the Ellerman Collection at Stellenbosch University, South Africa.

Snout-vent length (SVL) was measured to the nearest 0.01 mm using digital callipers and only sexually mature individuals were analysed. Size at sexual maturity was determined at the SVL of the smallest reproductively active specimen captured.

Assessment of female specimens

Both ovaries were examined and reproductive status was assessed according to Van Wyk and Mouton (1998) by grouping individual females into four categories based on the appearance of follicles and developing embryos. The four categories were pre-vitellogenic (for translucent, unyolked follicles of 2 mm in diameter or less), early vitellogenic (for yolked follicles between 2 mm and 5 mm in diameter), late vitellogenic (for yolked follicles 5-7 mm in diameter), and gravid. The ovaries of several non-gravid females were also assessed for evidence of a recent clutch. Embryonic development of gravid females was staged according to Dufaure and Hubert (1961).

To quantify female reproductive output, reproductive volume (Ramirez-Sandoval 2006) was calculated. Reproductive volume was defined as the volume of all yolked follicles or embryos present within an individual female. All yolked follicles or embryos within an individual female are of the same size or stage. Thus, reproductive volume was calculated by measuring the longest and shortest diameters of a yolked follicle or embryo to the nearest 0.01 mm using digital callipers. These values were then used to calculate the volume of a single follicle or embryo by using the formula for an ellipsoid: $V = 4/3\ \pi\ a^2b$, where V is volume, *a* is equal to half the shortest diameter and *b* is equal to half the longest diameter (Selby 1965). The resulting value for one yolked follicle or embryo was multiplied by the number of yolked follicles or embryos present in the female to give an overall reproductive volume. The same process was utilised for calculating follicular volume (using only vitellogenic follicles) and embryonic volume. Oviductal

eggs were counted for the evaluation of clutch size. Fat bodies were removed, dried to a constant mass, and weighed to the nearest 0.0001 g.

During the study three captured females gave birth. These individuals were used to calculate a body size index for the mother versus the offspring. This body size index was measured as the percentage of the average neonate (newborn) SVL to the mother's SVL.

Statistical analysis

All statistics were performed using the SPSS (14th edition) software package. A value of $P < 0.05$ was considered significant. All data were assessed to ensure that the appropriate statistical tests were performed and data were transformed where appropriate. Standard error values are always stated.

Spearman's Rank Order Correlation analysis was performed to test for the effect of SVL on reproductive volume, clutch size and fat body mass and also to establish the relationship between fat body mass and follicular volume, embryonic volume, reproductive volume, and monthly mean temperature and total monthly precipitation values. Pearson's Correlation Coefficient analysis was used to investigate the relationship between reproductive volume and precipitation and temperature. Pearson's Correlation Coefficient analysis was also used to establish the relationship between SVL and follicular and embryo volume. Analysis of Covariance, with SVL as covariate, was used to determine the monthly variation in reproductive volume, follicular volume, embryonic volume and clutch sizes with Bonferroni post-hoc tests to distinguish significant differences among months. A

Kruskal-Wallis analysis and Dunn's post-hoc tests were used to test if fat body mass showed any monthly variation and if fat body mass was different between reproductive stages.

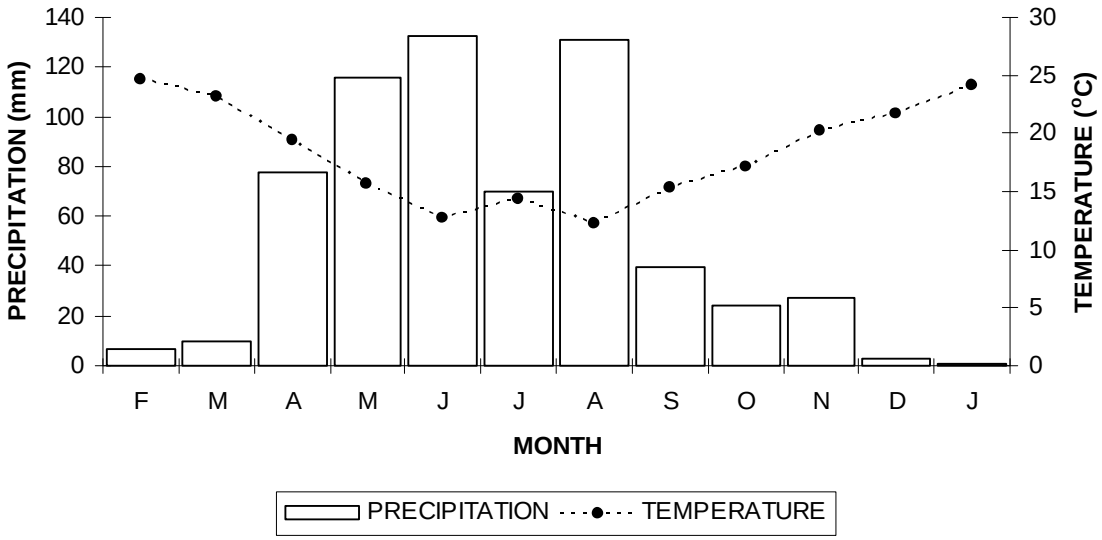


Figure 2.1 Mean monthly temperatures and monthly precipitation for the study area in the Western Cape, South Africa, from February 2005 until January 2006. Weather station data were combined and the means are plotted.

Figure 2.2 Image of the south-western tip of South Africa (Google Earth) showing study sites (white circles) and approximate distribution of *Bradypodion pumilum* (dotted line).

RESULTS

Eighty eight females were collected throughout the study period. Sexual maturity was determined at a SVL of 53 mm as the smallest female sampled with vitellogenic follicles measured 53.2 mm SVL. Five females in the sample were considered juveniles and excluded from further analysis. A range of female body sizes were present in each monthly sample to ensure an accurate representation of the reproductive population. During the full duration of the study 24 % of females sampled were pre-vitellogenic, 23 % were early vitellogenic, 13 % were late vitellogenic and 40 % were gravid.

Female *B. pumilum* do not exhibit a distinct seasonal pattern of reproductive activity and hence individual females are not completely synchronised (Figure 2.3). Gravid individuals also show a lack of synchrony in embryo stage amongst females (Figure 2.4).

Clutch size showed a significant positive relationship with SVL of adult females (Spearman's Rank Order Correlation; $r = 0.500$, $P < 0.01$; Figure 2.5). Mean monthly clutch size values did not vary significantly throughout the year (ANCOVA; $F = 0.978$, $P > 0.05$). No evidence of degenerating corpora lutea were found in the non-gravid females assessed.

Reproductive volume showed a significant positive relationship with female SVL (Spearman's Rank Order Correlation; $r = 0.386$, $P < 0.01$). Mean monthly values of reproductive volume varied significantly throughout the year (ANCOVA; $F = 4.97$, $P < 0.001$; Figure 2.6). However, reproductive volume is not significantly correlated

to monthly mean temperature values (Pearson's Correlation Coefficient; r = -0.141, P > 0.05) or monthly precipitation values (Pearson's Correlation Coefficient; r = 0.160, P > 0.05). Follicular volume showed a significant positive relationship with SVL (Pearson's Correlation Coefficient; r = 0.387, P < 0.05). Mean monthly values of follicular volume significantly differed throughout the year (ANCOVA; F = 5.33, P = 0.001; Figure 2.7). Embryonic volume showed a significant positive relationship with SVL (Pearson's Correlation Coefficient; r = 0.474, P < 0.01) but mean monthly values of embryonic volume did not differ significantly throughout the year (ANCOVA; F = 1.58, P > 0.05).

Fat body mass was not significantly correlated to female SVL (Spearman's Rank Order Correlation; r = 0.132, P > 0.05). Mean monthly values of fat body mass varied significantly throughout the year (Kruskal-Wallis Test; H = 43.4, df = 11, P < 0.001; Figure 2.8).

There was a significant negative correlation between reproductive volume and fat body mass (Spearman's Rank Order Correlation; r = -0.305; P < 0.05; Figure 2.9). Follicle volume (Spearman's Rank Order Correlation; r = 0.128, P > 0.05) and embryo volume (Spearman's Rank Order Correlation; r = 0.091, P > 0.05) were not significantly correlated to fat body mass. Fat body mass varies significantly between different reproductive stages (Kruskal-Wallis Test; H = 16.4, df = 3, P < 0.01; Figure 2.10). Fat body mass does not significantly correlate to monthly mean temperature values (Spearman's Rank Order Correlation; r = 0.476, P > 0.05) or total monthly precipitation (Spearman's Rank Order Correlation; r = -0.245, P > 0.05).

The average neonate SVL was 36.9 % of the mother's SVL.

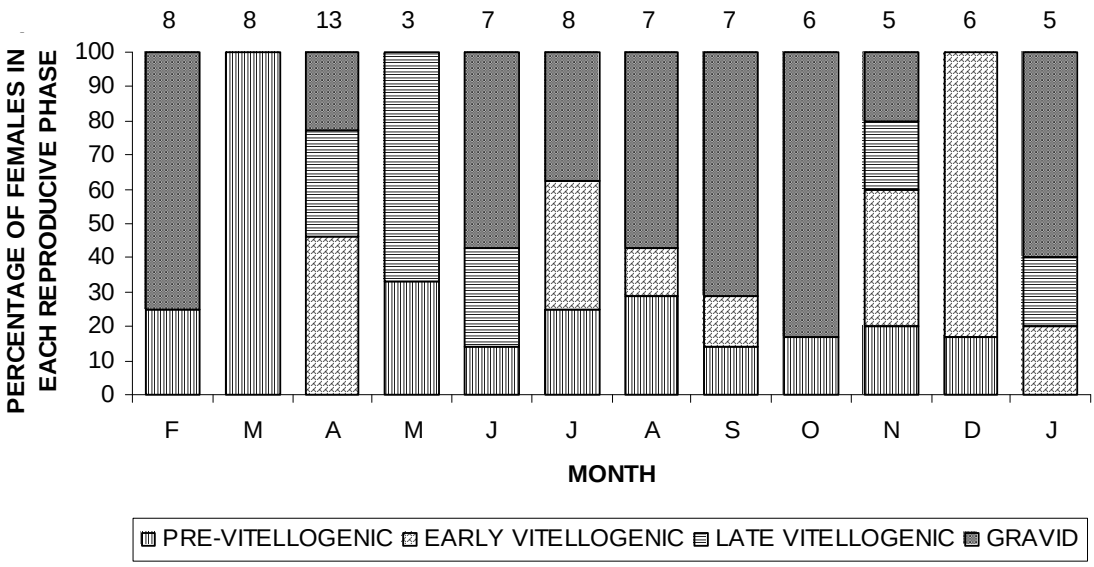


Figure 2.3 Column chart illustrating the reproductive phases of female *Bradypodion pumilum*, from February 2005 until January 2006. Monthly sample sizes are shown above columns.

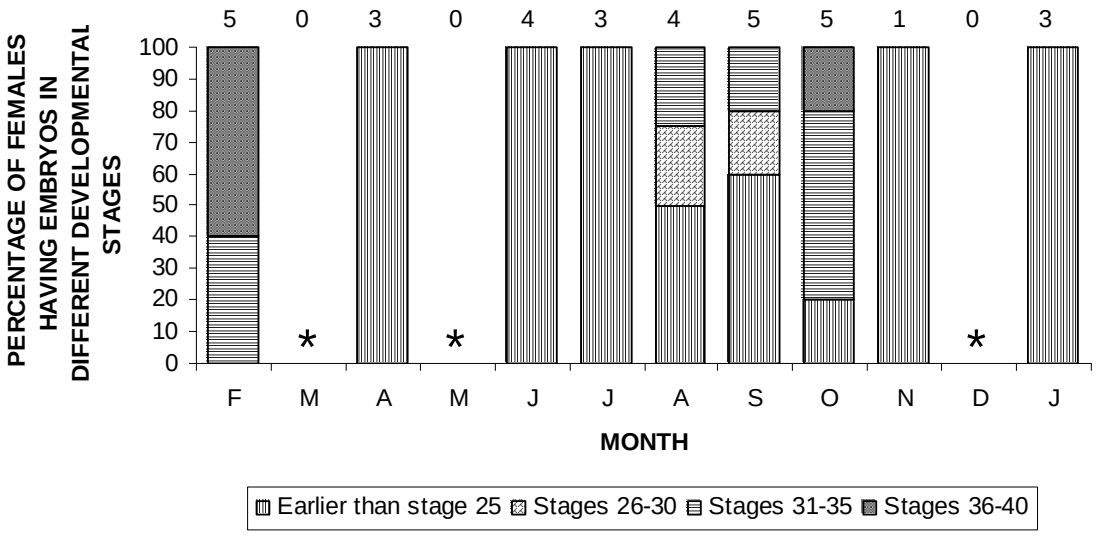


Figure 2.4 Column chart illustrating the developmental embryo stages of the gravid females of *Bradypodion pumilum*, from February 2005 until January 2006. Monthly sample sizes are shown above columns. The asterisks denote months where females collected were not gravid.

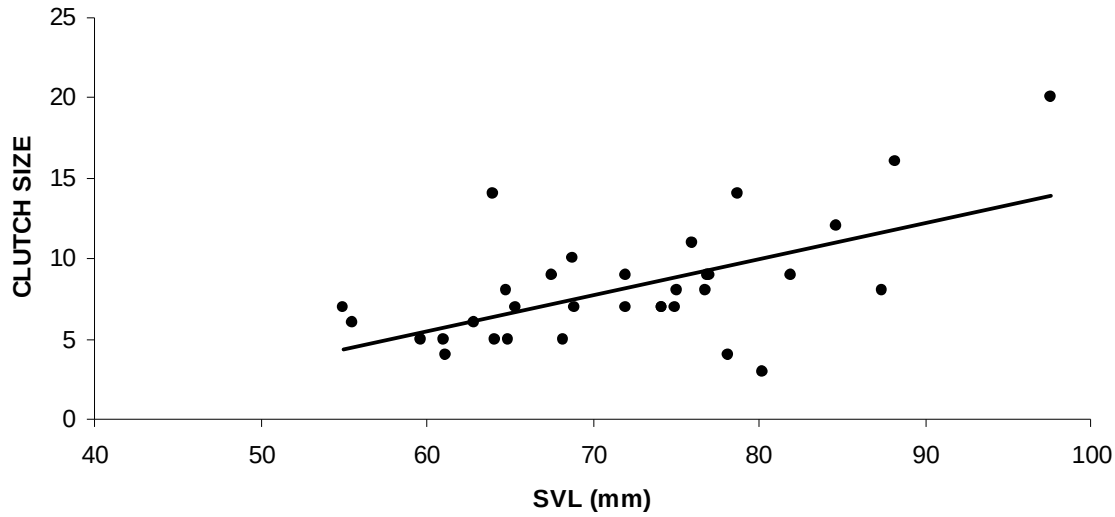


Figure 2.5 Scatterplot showing the relationship between female SVL and clutch size of *Bradypodion pumilum*.

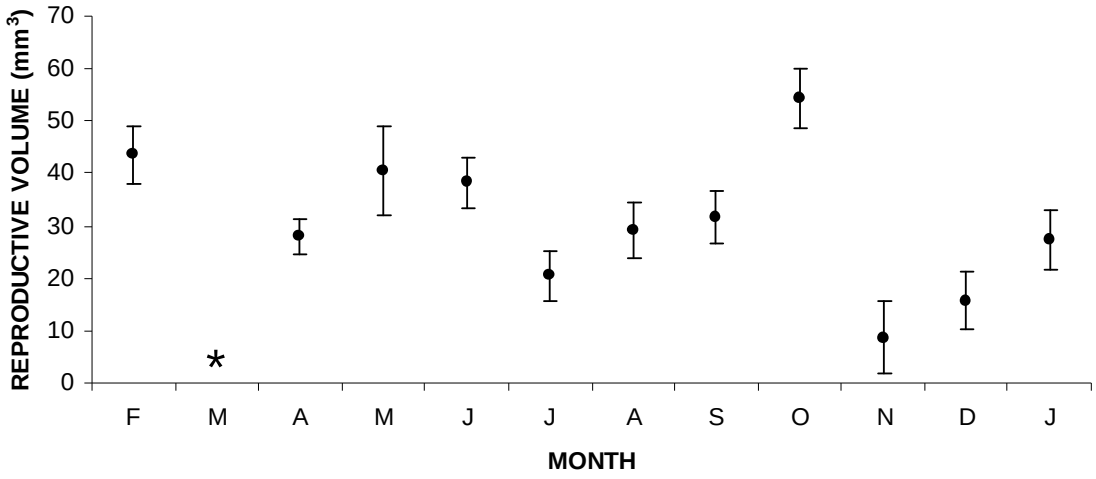


Figure 2.6 Line graph showing the variation in reproductive volume of *Bradypodion pumilum* females from February 2005 until January 2006. Size adjusted means and standard errors are plotted. Post-hoc tests reveal that February is different to November ($P < 0.05$) and October is significantly different to April, July, November and December ($P < 0.01$). The asterisk denotes month where only pre-vitellogenic females were present in the sample.

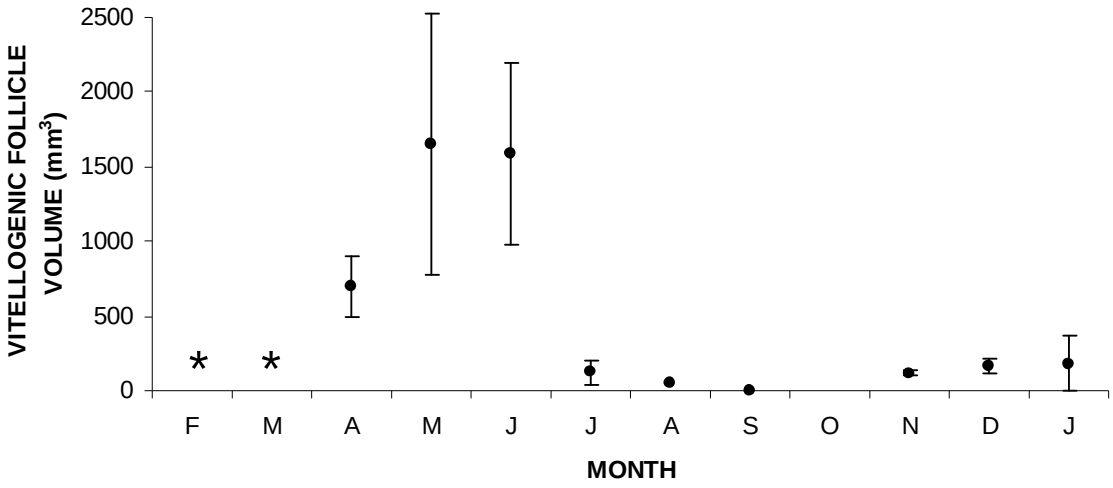


Figure 2.7 Line graph showing the variation in vitellogenic follicle volume of *Bradypodion pumilum* females from February 2005 until January 2006. Post-hoc tests reveal that September is different to April, May and June ($P < 0.05$). Mean values and standard errors are plotted. The asterisks denote months where no vitellogenic females were present in the sample.

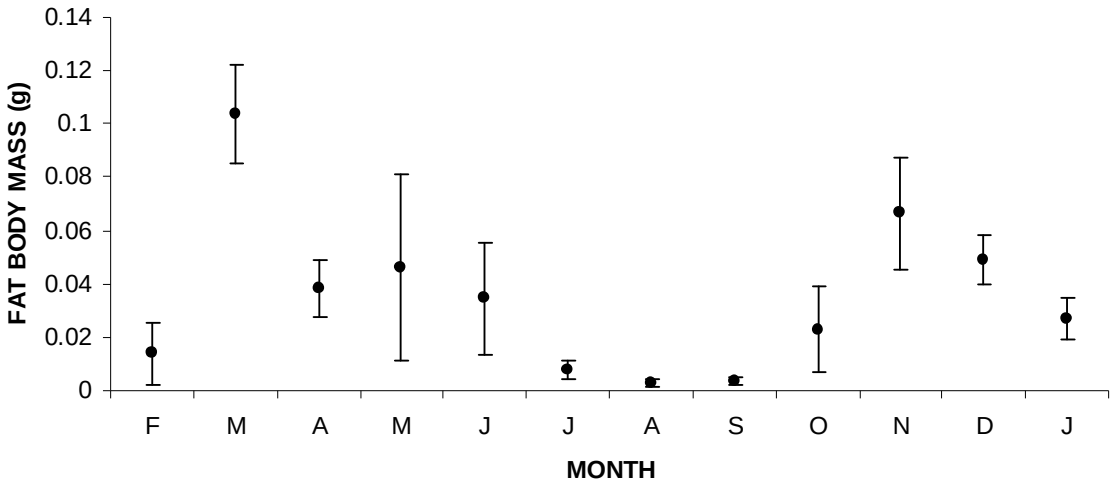


Figure 2.8 Line graph showing the variation in fat body mass of *Bradypodion pumilum* females from February 2005 until January 2006. Dunn's post-hoc tests show that March is significantly different to February, July, August and September ($P < 0.05$).

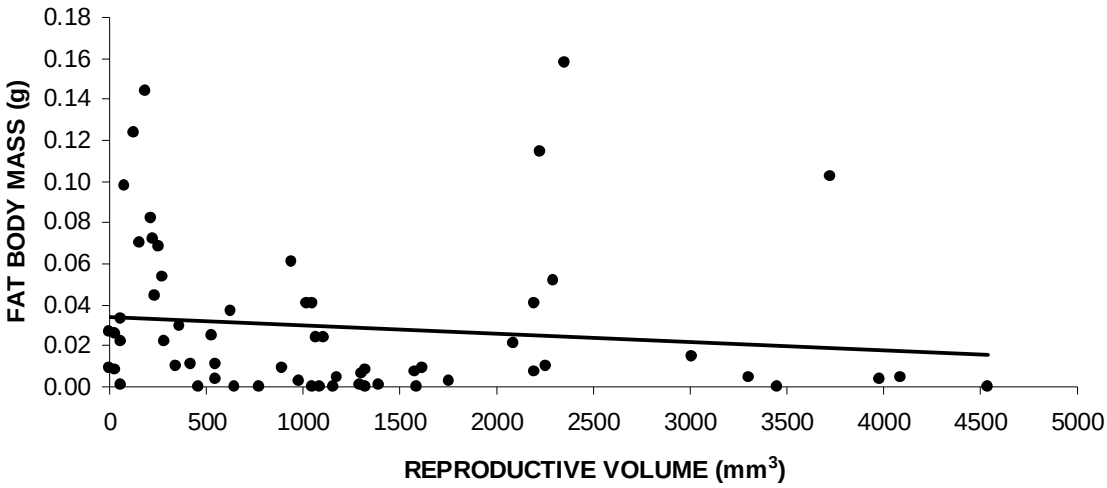


Figure 2.9 Scatterplot showing the relationship between female reproductive volume and fat body mass of *Bradypodion pumilum*.

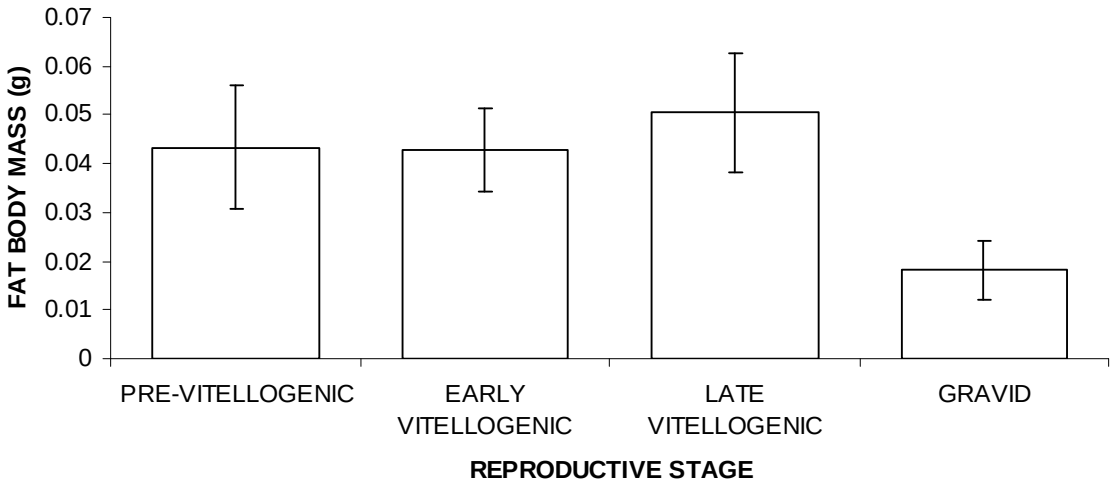


Figure 2.10 Chart showing mean and standard error of fat body mass in different stages of the reproductive cycle of female *Bradypodion pumilum*. Dunn's post-hoc tests reveal that gravid females are significantly different to both early vitellogenic and late vitellogenic females ($P < 0.05$).

DISCUSSION

Reproductive parameters of female Bradypodion pumilum

In temperate environments, some resources are seasonally limited and are known to have considerable effects on reptilian reproductive cycles (Licht 1984; Dunham *et al*. 1988; Diaz-Paniagua *et al*. 2002). Reptiles usually exhibit strict seasonal reproductive cycles in these environments. Seasonal cues can initiate various stages of the female reproductive cycle, such as, vitellogenesis, ovulation and parturition.

Female *Bradypodion pumilum* seem unaffected by such seasonal cues despite inhabiting a strictly seasonal area of southern Africa, with females reproducing asynchronously and relatively aseasonally. Conversely, low fat body mass values around late winter to early spring in this species suggest a seasonal fat body cycle with a possible decrease in resource availability at this time. Western Cape cordylids also show seasonal fat body cycles but unlike *B. pumilum*, they store greater reserves in winter and spring (Flemming and Van Wyk 1992; Flemming and Mouton 2002). However, as reproductive volume increases, fat body mass decreases in *B. pumilum* and a large number of the females collected at this time were gravid. Gravid individuals were also shown to have smaller fat bodies than individuals in any other reproductive stage. The high percentage of gravid females present throughout the year and anecdotal information on captive chameleons suggests the possibility of individual females producing multiple clutches per year (Busack and Busack 1967; pers. obs.).

The results in this study differ to a study by Burrage (1973) on the ecology of *B. pumilum*. From Burrage (1973) it is difficult to correctly interpret the female reproductive cycle of *B. pumilum* as births are said to occur from February to May and September, November and December but gravid individuals are found January to March, June to October and December. It remains unclear from Burrage (1973) if *B. pumilum* exhibit an aseasonal reproductive cycle, with females in all stages of the reproductive cycle present throughout the year. However, he did conclude that some seasonality is observed in clutch size with clutches in December larger than clutches in April. The present study found no evidence to support that statement. Furthermore, Burrage (1973) suggests follicles are yolked to 4 mm in diameter but halted if the individual is gravid, again no evidence of this was found in the present study. The inconsistency between the two studies could be explained by different sampling and analytical methods but also that specimens collected by Burrage (1973) are from localities that are now known to be within the range of two different species of dwarf chameleon. Specimens used in Burrage (1973) were collected from Port Nolloth (within the range of *B. occidentale*), Leeu Gamka and Beaufort West (within the range of *B. ventrale*). These species cover different biomes, and temperature and precipitation regimes to *B. pumilum* and their reproductive strategies are relatively unknown.

The following conclusions however, may be drawn from both studies; female *B. pumilum* show reproductive aseasonality, relatively high clutch sizes, asynchrony among individual females and the possibility of multiple clutches per year. These reproductive parameters are highly unusual for small, viviparous, temperate-zone lizards (Licht 1984; Duvall *et al.* 1982; James and Shine 1985).

It is thought that *B. pumilum* are highly preyed upon by a range of taxa due to their small size and arboreal lifestyle. Known predators include spiders, snakes, birds and domestic cats (Burrage 1973; Branch 1998; Tolley and Burger 2007). Both adults and juveniles are thought to be susceptible and it is possible that the high reproductive output exhibited by female *B. pumilum* is to counteract this high vulnerability to predation. The East African *Chamaeleo hoehnelii* is also a small, aseasonally reproducing, viviparous chameleon that produces two clutches per year (Lin and Nelson 1981). The reason suggested for the high reproductive output in *C. hoehnelii* is high predation rates (Lin and Nelson 1981).

Reproductive parameters in a fire-driven habitat

Reproductive aseasonality in reptiles is generally considered a tropical phenomenon (Licht 1984). Conversely, *B. pumilum* show reproductive aseasonality and inhabit a strictly seasonal area. It is possible that the reproductive aseasonality observed in *B. pumilum* may be in response to inhabiting an unpredictable fire-prone habitat.

The fynbos vegetation of the Cape Floristic Region burns between four and 45 years (Cowling and Richardson 1995). *Bradypodion pumilum* appear to be a particularly fire sensitive species and posses little fire avoidance strategies. Although fleeing or sheltering ability of *B. pumilum* has yet to be studied it is thought that they would suffer great losses in the event of a fire. Arboreal lizards may attempt to flee from fire rather than seeking alternative shelter sites to the vegetation usually utilised (Du Toit 2001). In addition to fleeing from the fire *B. pumilum* may actively avoid areas of old veld that may be susceptible to burning

thus avoiding the fire. Anecdotal observations suggest that *B. pumilum* are absent (or in very low densities) in older vegetation (pers. comm. D. Houniet and K. Hopkins). However, the reason for this absence in old veld remains to be studied.

Even if an individual survives the blaze, the post-fire conditions are thought to be particularly harsh for ectotherms. With the vegetation decimated there is a severe lack of shelter from both abiotic conditions and possible predators, and the possible food supply is thought to fluctuate greatly after a fire (Warren *et al.* 1987; Mushinsky 1992; Whelan 1995; Cavitt 2000). Chameleons appear particularly vulnerable to this post-fire environment due to their heavy reliance on the vegetation because of their cryptic nature. This reliance on the vegetation appears especially evident in *B. pumilum* with the possibility of three distinct morphotypes that correspond to three different vegetation types (pers. comm. K. Hopkins).

In addition to survival in the post-fire habitat it may be difficult for females to find a mate after a fire or migrate to a more favourable neighbouring habitat. The home range size of *B. pumilum* has not previously been studied but it is thought that they are not especially mobile. With low mobility it is likely that survivors of the blaze repopulate the area. If females are unable to find a mate in the post-fire environment it is possible that an isolated female *B. pumilum* is already gravid regardless of the time of year. This reproductive cycle ensures that the entire reproductive effort in a single year is not wasted in the event of a fire. Anecdotal observations also suggest that female *B. pumilum* isolated from males are able to reproduce via stored sperm (Atsatt 1953) thus helping an isolated female reproduce after a fire.

Chamaesaura anguina, a viviparous grass lizard from the Western Cape, South Africa also inhabits the pyrophillic fynbos. It is known to be a fire-sensitive species with high direct mortality and low post-fire survival (Du Toit 2001). Similar to *B. pumilum*, *C. anguina* exhibits an aseasonal female reproductive cycle and high clutch sizes; highly unusual for the Cordylidae (Du Toit *et al.* 2003). It is suggested that this reproductive strategy is advantageous to *C. anguina* to help ensure rapid recruitment after a fire (Du Toit *et al.* 2003).

It appears that aseasonal reproduction and high fecundity in the arboreal lizards inhabiting the fire-driven fynbos of the Cape Floristic Region may be a common trend but more study is needed for clarification. Studying the reproductive cycle of species closely related to both *B. pumilum* and *C. anguina that* do not inhabit fire-driven vegetation, would increase our understanding of this unusual reproduction. Catling and Newsome (1981) showed that mammals in fire-prone areas are aseasonal reproducers whilst other closely related species occurring outside of the fire-prone areas are seasonal.

Reproductive aseasonality within arboreal lizards in the Western Cape may be linked to inhabiting a fire-prone habitat. An aseasonal female reproductive cycle with high clutch sizes ensures the yearly reproductive effort is not lost in a single fire event, increases the likelihood of a female surviving the fire being gravid and ensures rapid recruitment to the population after a fire. Even if this unusual reproductive cycle is not an adaptation to an unpredictable habitat, it appears beneficial in this type of changeable environment.

CONCLUSION

Female *Bradypodion pumilum* show unusual reproductive characteristics for a viviparous lizard inhabiting a temperate environment. They are aseasonal and asynchronous between individual females, have high clutch sizes and it is likely that they produce multiple clutches per year. *Bradypodion pumilum* are small, arboreal lizards with a high reproductive output, which may be due to a high vulnerability to predation and fire. However, to completely understand the reproductive strategy of *B. pumilum* the male cycle and sperm storage (both male and female) must be determined. Knowledge of the male cycle would establish if males are synchronous and seasonal, and when and how often mating may take place. It would also be valuable to investigate sperm storage in *B. pumilum* as knowing if females can reproduce in absence of a male could help to understand whether a female isolated after a fire is able to reproduce without the need for a male. With more information and a greater understanding of the reproductive strategy of *B. pumilum* and related species, possible drivers for the evolution of this unusual female reproductive cycle (and possibly the species as a whole), may be revealed.

CHAPTER THREE

REPRODUCTIVE PARAMETERS OF THE MALE CAPE DWARF CHAMELEON, *BRADYPODION PUMILUM*

INTRODUCTION

Male reproductive cycles

Seasonal fluctuations in resource availability may have an effect on reptile spermatogenic cycles (Licht 1967a; 1967b). However, tropical males may produce sperm all year round whilst temperate males have a period of reproductive inactivity. In temperate regions, seasonal variation in climatic conditions such as temperature, precipitation and photoperiod may initiate different stages of the spermatogenic cycle (Licht 1967a; 1967b). The seasonality of biotic factors such as food availability may influence the accumulation of fat body reserves thereby indirectly affecting the spermatogenic cycle. There are two types of seasonal spermatogenic activity, pre-nuptial, where sperm is produced before mating (autumn/winter) and regression takes place in summer, and post-nuptial, where sperm is produced after mating (spring/summer) and stored in the male vas deferens or epididymis, until the following mating season (Flemming 1993a; 1993b; Flemming 1994; Flemming and Bates 1995; Van Wyk 1995; Van Wyk and Mouton 1996; Van Wyk and Mouton 1998; Flemming and Mouton 2002; Du Toit *et al.* 2003. Temperate zone lizards are usually pre-nuptial whilst a post-nuptial cycle is generally observed in snakes and chelonians (Licht 1984).

Chameleon reproductive cycles

Previous studies on chameleon reproduction have focused on factors specifically affecting the female reproductive cycle (Menzies 1958; Cuadrado and Loman 1999; Diaz-Paniagua *et al.* 2002) but few concentrate on male testicular cycles and how it relates to the female cycle. Special emphasis is placed on females as the bearers of offspring, however, an adequate understanding of the male cycle is

important in understanding species' reproductive strategies, although examples in the literature are scarce.

The Cape Dwarf Chameleon, **Bradypodion pumilum**

The Cape Dwarf Chameleon, *Bradypodion pumilum*, is a small, arboreal, insectivorous, viviparous dwarf chameleon from the Western Cape, South Africa (Branch 1998; Tolley and Burger 2007). *Bradypodion pumilum* may be found in a variety of vegetation such as, renosterveld, strandveld, fynbos but they appear successful in urban gardens and parks (Branch 1998; Tolley and Burger 2007). Female *B. pumilum* show an aseasonal reproductive cycle, with high clutch sizes and it is likely that multiple clutches are produced per year (Chapter 2).

AIM

Burrage (1973) made anecdotal observations on the male reproductive cycle of *B. pumilum*; stating that the testes are inactive in June and July. These observations are inadequate to accurately assess the reproductive strategy of *B. pumilum*. Therefore, the aim of this chapter is to describe the male spermatogenic cycle of *Bradypodion pumilum*, its relation to seasonality and the female cycle of *B. pumilum,* and hence the mating strategy of *B. pumilum*.

Due to the extraordinary female cycle and inhabiting a fire-prone area, I hypothesise that male *B. pumilum* will produce sperm for either prolonged periods or aseasonally.

MATERIALS AND METHODS

Study site and collection of specimens

The Western Cape of South Africa has a Mediterranean climate characterised by high winter rainfall and a dry summer period (Schulze 1997; Midgley *et al.* 2005). Average monthly temperatures in degrees º C (weather stations Strand: 0005609 8 and Paarl: 0021823 0), and monthly rainfall in mm (weather stations Strand: 0005609 8, Paarl: 0021823 0, Stellenbosch: 0021656A6 and Somerset West: 0005603 7) were obtained from weather stations close to the study area, from February 2005 until January 2006. The weather station data were used to calculate overall monthly means of temperature and rainfall for the study period (Figure 2.1). Weather station data were obtained from WeatherSA.com.

Male *Bradypodion pumilum* specimens were collected in monthly samples from Stellenbosch (3318DD) and Somerset West (3418BB), between February 2005 and January 2006 (Figure 2.2). Stellenbosch and Somerset West were chosen as sites because of the semi-natural, relatively stable vegetation found there. The lizards were sacrificed within 24 hours of capture, fixed in 10 % formalin and preserved in 70 % ethanol. The specimens were deposited in the Ellerman Collection at Stellenbosch University, South Africa.

Snout-vent length (SVL) was measured to the nearest 0.01 mm using digital callipers and only sexually mature individuals were analysed. Size at sexual maturity was determined at the SVL of the smallest reproductively active (presence of spermatozoa in the epididymis or testis) specimen captured.

Assessment of male specimens

The left testis was removed and the longest and shortest diameter of the testis was measured to the nearest 0.01 mm using digital callipers. These measurements were used to calculate testicular volume using the formula for an ellipsoid; $V = 4/3\ \pi\ a^2\ b$ where V equals volume, *a* is half the shortest diameter and *b* is half the longest diameter (Selby 1965).

The testes were dehydrated, cleared with Xylene, embedded in paraffin wax and sectioned at 5-7 µm. Sections were stained using Ehrlich's Haematoxylin and Eosin and digital photographs were taken of the sections (QWin software package, Leica). Diameters of ten seminiferous tubules and ten Leydig cells were measured for each specimen and presence of spermatozoa in the epididymis was examined. Seasonal histological changes in the testis were recorded qualitatively using the methods of Licht (1967ab), Mayhew (1971) and Van Wyk (1995).

For 44 males the epididymis was removed, dehydrated, cleared with Xylene, embedded in paraffin wax and sectioned at 5-7 µm. Sections were stained using Ehrlich's Haematoxylin and Eosin and digital photographs were taken of the sections (QWin software package, Leica). These sections were then examined under a light microscope to establish presence or absence of sperm in the epididymis.

Fat bodies were removed, dried to a constant mass, and weighed to the nearest 0.0001 g.

Statistical analysis

All statistics were performed using the SPSS (14th edition) software package. A value of $P < 0.05$ was considered significant. Data were assessed for a normal distribution and transformed where appropriate. Standard error values are always stated.

To establish the relationship between testis volume and both adult male SVL and fat body mass a Spearman's Rank Order Correlation was used. Pearson's Correlation Coefficients were used to establish the relationship between both mean testis volume and fat body mass with both mean monthly temperature and total monthly precipitation.

Analysis of Covariance with Bonferroni post-hoc tests were used to establish if mean monthly values of testis volume varied and if testes volume differed with different spermatogenic stages. Analysis of Variance and Tukey's post-hoc tests were used to establish the variation in mean monthly values of seminiferous tubule diameters, mean monthly values of Leydig cell diameters and mean monthly SVL values. Analysis of Variance was also used to test for the variation of SVL between spermatogenic stages.

The fat body data were subjected to a Kruskal-Wallis test and Dunn's post-hoc tests to establish if a significant difference occurred in monthly mean values during the study period. A Kruskal-Wallis test and Dunn's post-hoc tests were also used to test for differences in fat body mass at different stages of the spermatogenic cycle.

RESULTS

Seventy-five males in total were collected between February 2005 and January 2006. A range of male body sizes were present in each monthly sample but no one month contained significantly larger or smaller individuals than any other to ensure an accurate representation of the reproductive population (ANOVA; $F = 1.734$, $P < 0.05$). The smallest reproductively active male, determined by presence of spermatozoa in the epididymis or testis, had a SVL of 41.7 mm. There were no smaller males collected and hence no specimens were considered juveniles.

A statistically significant positive relationship was found between testis volume and male SVL (Spearman's Rank Order Correlation; $r = 0.642$, $P < 0.001$; Figure 3.1). Testis volume did not differ significantly when compared across different stages of the spermatogenic cycle (ANCOVA; $F = 1.65$, $P > 0.05$) as a range of testis sizes were seen throughout the year and at different spermatogenic stages (Figure 3.2A). Mean monthly values of testicular volume differed significantly throughout the year (ANCOVA; $F = 3.82$, $P < 0.001$; Figure 3.2B). No significant correlation occurs between mean monthly testis volume (adjusted for male size) and temperature (Pearson's Correlation Coefficient; $r = -0.460$, $P > 0.05$) or mean monthly testis volume values and precipitation (Pearson's Correlation Coefficient; $r = 0.344$, $P > 0.05$).

Mean monthly values of seminiferous tubule diameters differed significantly (ANOVA; $F = 12.8$, $P < 0.001$; Figure 3.3). Leydig cells were present throughout the study period and found to differ significantly in diameter (ANOVA; $F = 4.35$, $P < 0.001$; Figure 3.4).

Sperm was available throughout the year with peak spermatogenesis occurring from March to June and then again August to December however, some regression of the testis occurred mid year and again in January (Figure 3.5). The average SVL of males in each spermatogenic stage did not differ significantly (ANOVA; $F = 1.20$, $P > 0.05$).

Mean monthly values of fat body mass differed significantly with the largest monthly means found in October and smallest in August (Kruskal-Wallis Test; $H = 23.1$, $df = 11$, $P < 0.05$; Figure 3.6). Although fat body mass and testis volume appear to follow the same pattern no significant correlation was found (Spearman's Rank Order Correlation; $r = -0.002$, $P > 0.05$). Fat body mass differed significantly when compared across different stages of the spermatogenic cycle (Kruskal Wallis Test; $H = 11.7$, $df = 5$, $P < 0.05$; Figure 3.7). Fat body mass did not significantly correlate with temperature (Pearson's Correlation Coefficient; $r = 0.383$, $P > 0.05$) or precipitation (Pearson's Correlation Coefficient; $r = -0.349$, $P > 0.05$).

Of 44 sampled males, 81.8 % had sperm in the epididymis. More males had sperm present in the epididymis in autumn than any other time of year (Figure 3.8).

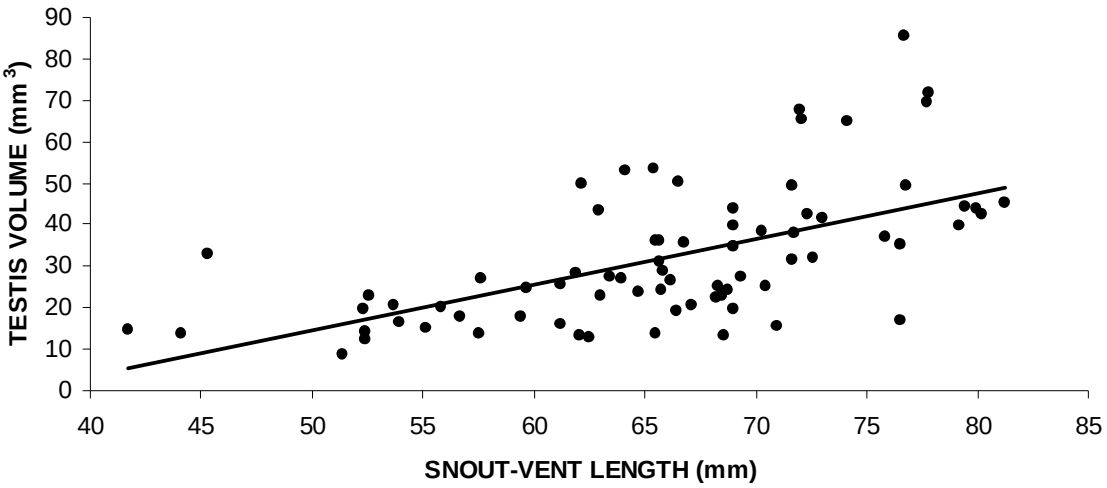


Figure 3.1 Scatterplot showing the relationship between male SVL and testis volume in *Bradypodion pumilum.*

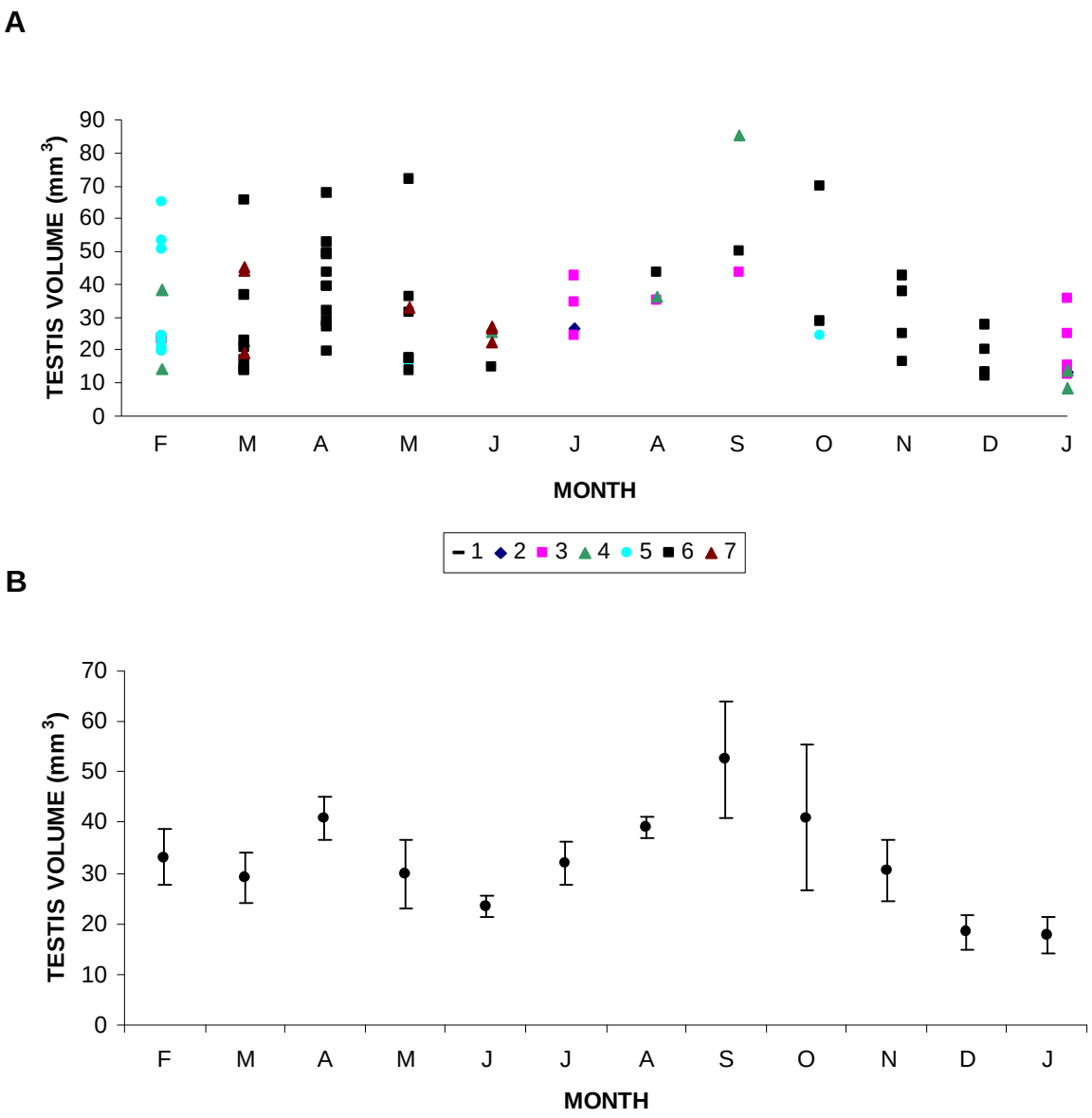


Figure 3.2 Variation in testis volume at different spermatogenic stages (A) and mean and standard error of testis volume (B) of male *Bradypodion pumilum* from February 2005 until January 2006. Stages 1-7 are defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced. January is significantly different to February, April, May and September ($P < 0.05$).

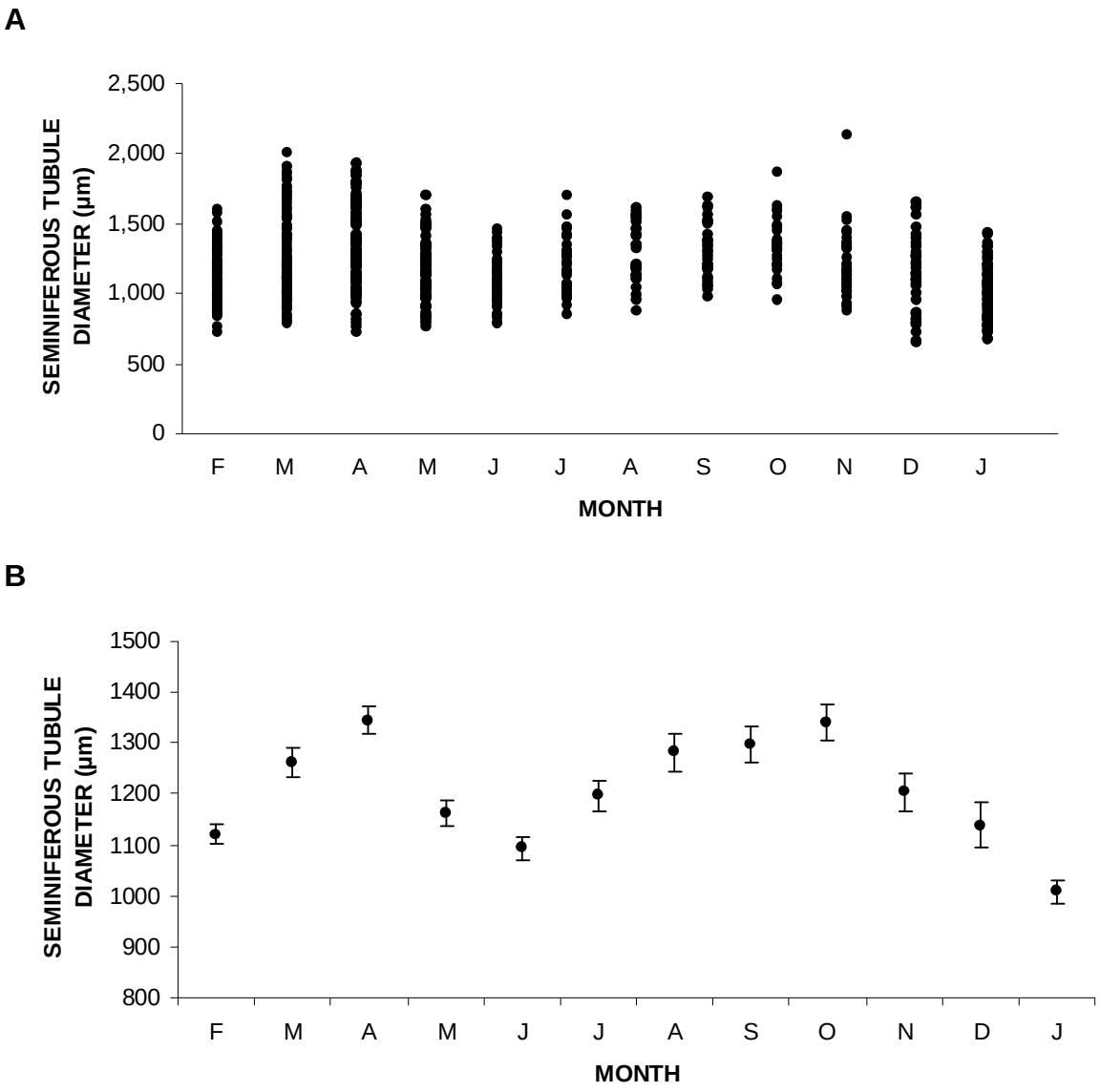
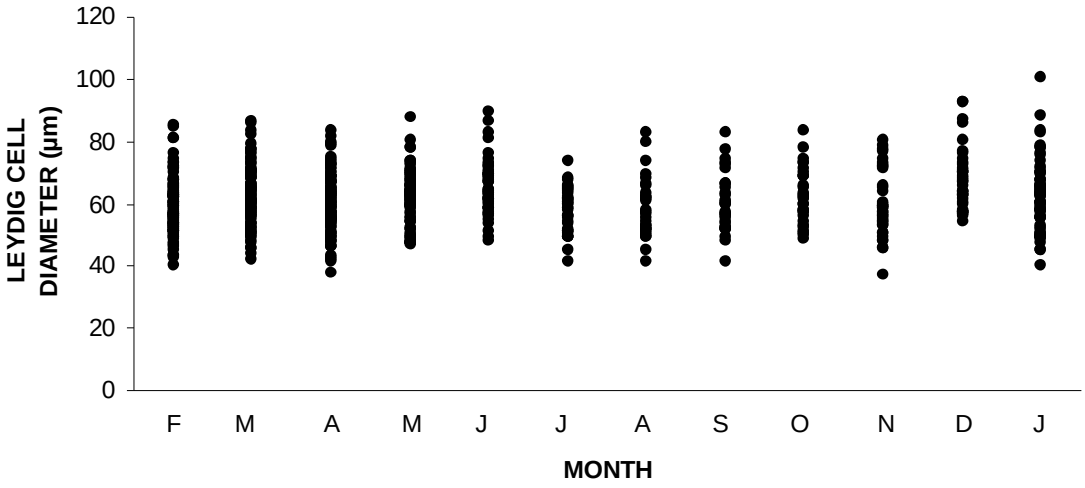


Figure 3.3 Variation in seminiferous tubule diameters in male *Bradypodion pumilum* (A) and mean and standard error of seminiferous tubule diameters (B) from February 2005 until January 2006. January is significantly different to February, March, April, May, July, August, September, October and November ($P < 0.05$). February is significantly different to March, April, August, September and October ($P < 0.05$). April is significantly different to May and December ($P < 0.001$). June is significantly different to March, April, August, September, October ($P < 0.05$) and October is significantly different to May and December ($P< 0.05$).

A



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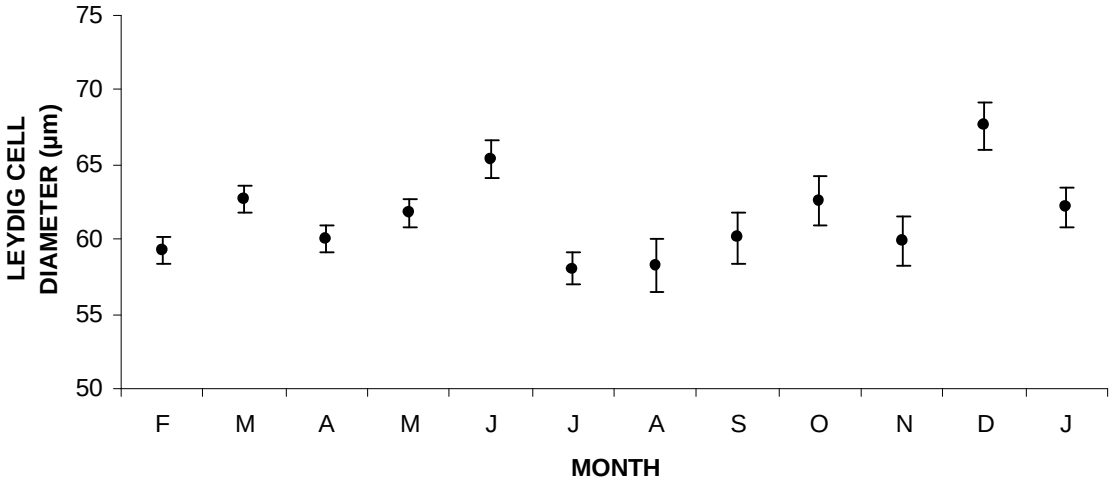

Figure 3.4 Variation in Leydig cell size (A) and mean and standard error (B) in male *Bradypodion pumilum* from February 2005 until January 2006. June is significantly different to February, April, July and August ($P < 0.05$). December is significantly different to February, April, July, August, September and November ($P < 0.05$).

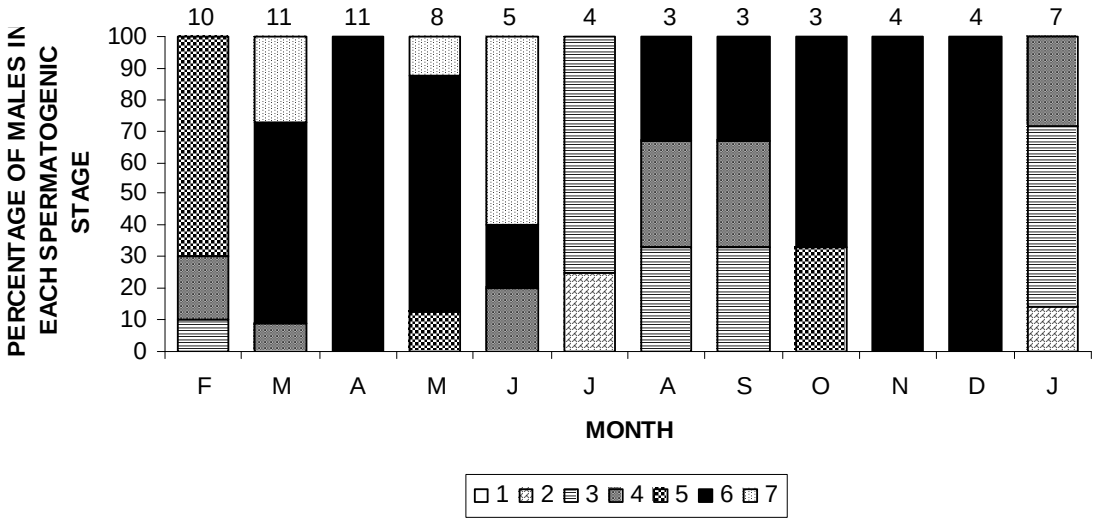

Figure 3.5 Column chart showing the variation in spermatogenic activity, amongst individual *Bradypodion pumilum* males from February 2005 until January 2006. Stages 1-7 are defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced.

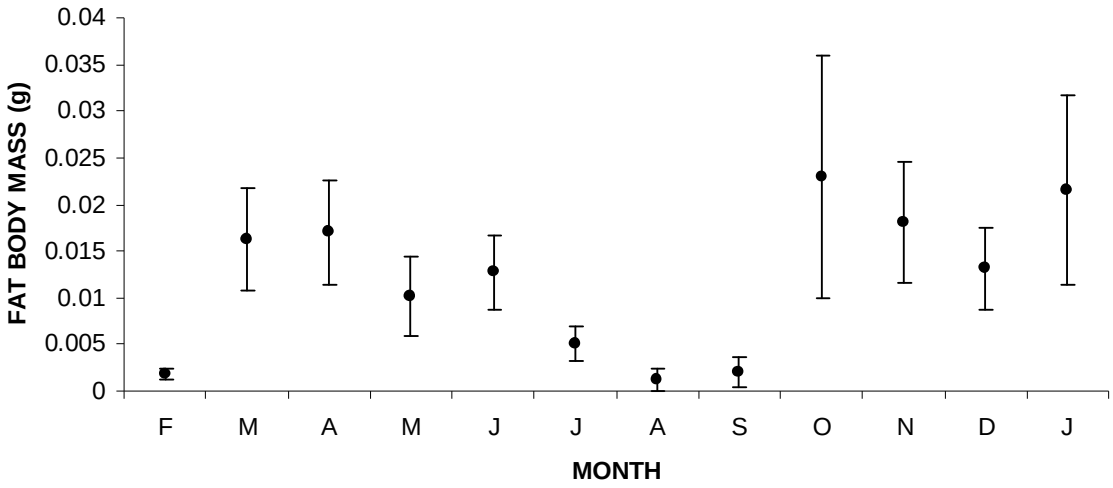

Figure 3.6 The variation in fat body mass in male *Bradypodion pumilum* from February 2005 until January 2006. Means and standard errors are plotted. Dunn's post-hoc tests did not reveal any significant differences among months.

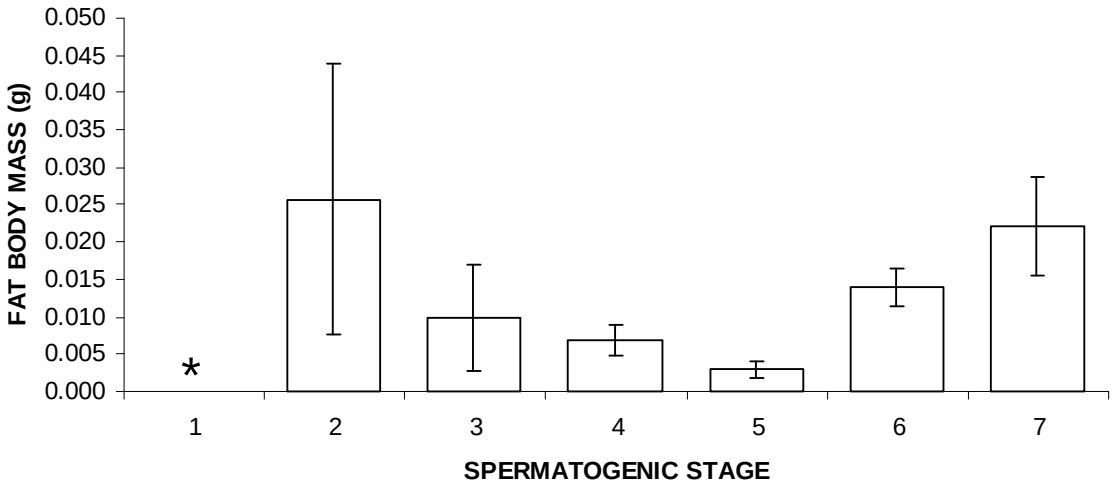

Figure 3.7 Chart showing the fat body mass at different stages of the reproductive cycle in *Bradypodion pumilum*. Means and standard errors are plotted. Stages 1-7 are defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced. The asterisk denotes spermatogenic stage not present in sampled males. Dunn's post-hoc tests did not reveal any significant differences among months.

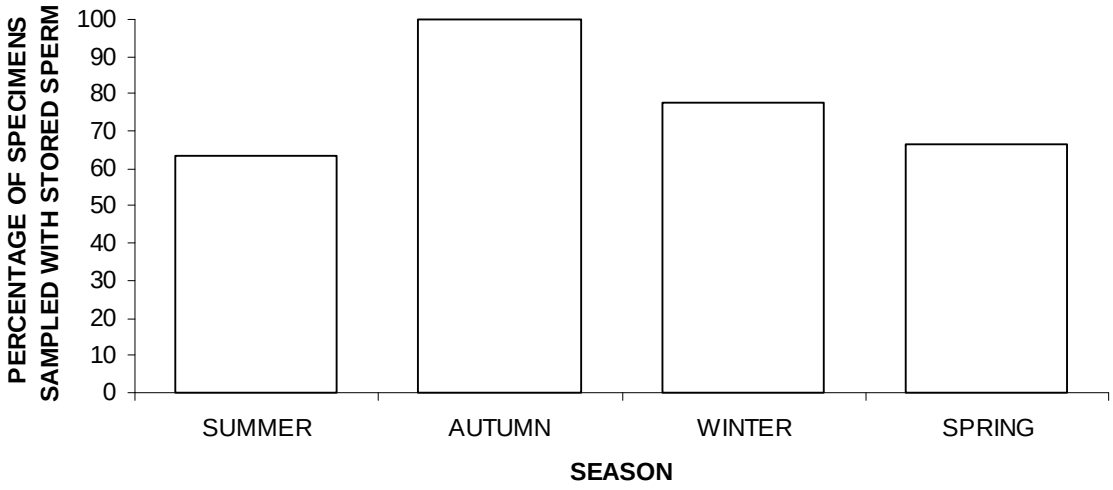

Figure 3.8 Chart showing percentage of *Bradypodion pumilum* males sampled with sperm present in the epididymis in the different seasons of the year.

DISCUSSION

Male reproductive cycle of Bradypodion pumilum

Male *B. pumilum* are able to reproduce throughout the year but there are two distinct periods of increased reproductive activity. The testicular cycle appears to be bimodal and has elements of both a pre-nuptial and a post-nuptial testicular cycle. This possible bimodal cycle is not an artefact of age as both large and small males were collected throughout the year, and represented a range of spermatogenic stages. The first peak in reproductive activity occurs in autumn and the second in spring/summer. Although males can reproduce all year round the two peaks suggests that male *B. pumilum* may have a modified seasonal cycle as opposed to a strictly aseasonal one where reproductive activity would be of a similar level throughout the year. However, sperm is available for the entire year even when sperm production is low (autumn/winter), maximum storage of sperm occurs at this time in the male epididymis, possibly due to the previous peak in reproductive activity. Fat body mass also shows two peaks, the first in autumn and the second in spring/summer with low values in winter/early spring. The low fat body values and the decrease in reproductive activity around winter may be an indication of limiting resources at this time.

The two peaks in reproductive activity appear to correspond to the environmental data even though no significant relationships were found; the first peak occurring just before the heavy rainfall starts and the second peak just after the heavy rainfall begins to decline. These peaks also appear to occur when the temperature is at a similar value i.e. high and low temperatures do not appear as favourable for reproductive activities as the temperature values in between.

Although it is not unusual for male reptiles to be able to reproduce throughout the year, it is unusual for constant spermatogenesis to occur in temperate-zone species where reproduction in other species is generally seasonal (Goldberg and Robinson 1979; Simbotwe 1980). *Tarentola mauritanica*, occurs in a Mediterranean environment with distinct seasonal periods and also shows a continuous spermatogenic cycle (Angelini *et al.* 1983). As in *B. pumilum*, *T. mauritanica* showed seasonal variation in the amount of sperm produced but the testes never completely regressed (Angelini *et al.* 1983). Consequently, it seems improbable that males can maintain a continuous spermatogenic cycle in a seasonal environment and hence be considered truly aseasonal.

Relation to the female cycle of **Bradypodion pumilum**

To understand the reproductive strategy of a species it is necessary to examine the male and female reproductive cycles together. In *B. pumilum*, males have sperm available and females are able to reproduce, throughout the year, clutch size is high, and it is likely that multiple clutches per year are produced (Chapter 2). *Bradypodion pumilum* appear to be able to maintain this high reproductive output despite a possible decrease in available resources, indicated by a decline in fat body mass in winter for both sexes (Chapter 2 and the present study). The life history characteristics of *B. pumilum* suggest that it is an r-selected species; early maturation, high mortality, continuous reproduction with high fecundity.

Although scientific studies are lacking, it is generally thought that both adult and juvenile *B. pumilum* are particularly vulnerable to predation by a range of taxa due to their lack of defence or fleeing ability. Predators include spiders, snakes, birds

and domestic cats (Burrage 1973; Branch 1998; Tolley and Burger 2007). Early maturation and the high reproductive output of *B. pumilum* may help to counteract this high mortality by predation. Lin and Nelson (1981) concluded that *Chamaeleo hoehnelii* is more highly preyed upon than *C. jacksonii*, another chameleon inhabiting the same area (Kenyan highlands), and this is reflected in their reproductive characteristics. Both chameleon species are viviparous but *C. jacksonii* is a later-maturing, seasonal breeder that produces a single clutch of larger offspring per year whilst *C. hoehnelii* is an earlier-maturing, aseasonal breeder, producing two clutches of smaller young per year.

Bradypodion pumilum is distributed in areas that are prone to burning. The fynbos occurring in the Cape Floristic Region (CFR), South Africa, generally burns between 4 and 45 years (Cowling and Richardson 1995). It is thought that *B. pumilum* is a fire-sensitive species and would suffer great losses in a blaze. In addition the post-fire habitat would be harsh. Individuals may be isolated for long periods of time whilst the vegetation recovers as it is thought that *B. pumilum* is not especially mobile. An aseasonal male and female reproductive cycle and the willingness to mate year round (large Leydig cells throughout the year) would be necessary. In addition, aseasonal reproduction increases the likelihood that a surviving (and possibly isolated) female will already be gravid and would not need to find a male to reproduce. Furthermore, in seasonally reproducing animals the entire reproductive effort for the year would be lost if a fire occurred during the breeding season, this is avoided in aseasonally reproducing animals. It would also benefit *B. pumilum* if females could store sperm as if they are isolated after a fire they could reproduce without need for a male.

Chamaesaura anguina, a grass lizard also inhabiting the fynbos of the CFR, also shows unusual reproductive characteristics; females are aseasonal, males have a post-nuptial spermatogenic cycle and fecundity is high (Du Toit *et al.* 2003). It is suggested that the reproductive strategy observed in *C. anguina* could be an adaptation to life in an unpredictable fire-prone habitat (Du Toit *et al.* 2003).

CONCLUSION

Bradypodion pumilum show unusual reproductive characteristics for a temperate-zone, viviparous lizard: an aseasonal female reproductive cycle with high fecundity and a prolonged male cycle with sperm available throughout the year. A viviparous, aseasonal, r-selected, temperate lizard species is extremely unusual. It is thought that these unusual reproductive characteristics may be in response to life in a fire-prone environment. Oviductal sperm storage would be an advantage to female *B. pumilum* isolated after a fire as they would be able to reproduce and repopulate the area in absence of a male until the vegetation recovers. However, no detailed study on sperm storage in female *B. pumilum* has concluded specialised sperm storage structures in the oviduct. It is therefore necessary to determine if *B. pumilum* possess specialised sperm storage structures in the oviduct, if females are able to store sperm for prolonged periods and if females are able to reproduce in absence of a male. To assess if this unusual reproduction is in response to a fire-prone habitat other species in the *Bradypodion* genus that do not inhabit fire-driven vegetation would have to be investigated to establish if they possess the same reproductive characteristics as *B. pumilum*.

CHAPTER FOUR

SPERM STORAGE IN FEMALE *BRADYPODION PUMILUM*

INTRODUCTION

Many reasons have been suggested for the evolution of female sperm storage in vertebrates. The first is the fertilisation of further clutches, where males may be a limited resource or population densities are low (Conner and Crews 1980). This hypothesis however, seems unlikely as the main reason for the evolution of female sperm storage since only the most dispersed and immobile species would struggle to find a mate (Pearce and Avise 2001). The second is to synchronise male and female reproductive cycles (Cohen 1977). If certain phases of the reproductive cycle are asynchronous then sperm storage may serve to synchronise these reproductive events (Cohen 1977). The third is for cryptic female choice as sperm storage may allow females to theoretically 'choose' the paternity of their offspring after copulation, i.e. by way of mate choice or sperm competition (Birkhead *et al.* 1993; Eberhard 1998). Finally, Conner and Crews (1980) suggest that sperm storage in multiple clutching lizards may be to fertilise subsequent clutches from a single mating, to minimise the cost of mating.

Sperm storage tubules have been found in many reptile families; in the infundibular region in the Agamidae, Anguidae, Eublepharidae, Gekkonidae, Iguanidae, Scincidae and in the vaginal region in the Agamidae, Chamaeleonidae, Polychrotidae and Iguanidae (for review see Sever and Hamlet 2002).

The Cape Dwarf Chameleon, Bradypodion pumilum

The Cape Dwarf Chameleon, *Bradypodion pumilum*, is a small, insectivorous, viviparous dwarf chameleon from the Western Cape, South Africa (Branch 1998; Tolley and Burger 2007). It inhabits a wide variety of vegetation types ranging from

urban parks and gardens to more natural vegetation such as the fire-driven fynbos, renosterveld and strandveld. Female *B. pumilum* show an aseasonal reproductive cycle, with high clutch sizes and it is likely that multiple clutches are produced each year (Chapter 2). Male *B. pumilum* are also able to reproduce throughout the year but show elements of a bimodal reproductive cycle with peaks in spermatogenic activity in autumn and spring/summer (Chapter 3). Veith (1974) concludes sperm storage in the oviduct of *B. pumilum.* However I inspected the photomicrographs of the alleged sperm storage receptacles, and they did not contain any sperm or specialised structures. It may be possible that what was deduced as sperm storage receptacles were in fact folds in the posterior oviduct. Atsatt (1953) also implies sperm storage in *B. pumilum* as a captive specimen became pregnant without mating although no histological evidence was presented.

AIM

In a species where both sexes reproduce aseasonally, specialised sperm storage structures in the posterior oviduct would appear unnecessary for the production of further clutches; providing there was high enough population densities and enough males. However, *B. pumilum* inhabit fynbos, a fire-driven vegetation. Individuals are known to be isolated for long periods in patches of fynbos after a fire (Du Toit 2001). As *B. pumilum* are arboreal, females are expected to be isolated for long periods of time whilst the vegetation recovers. Hence this chapter aims to establish if sperm storage occurs in female *B. pumilum*, specifically focussing on the presence and location of sperm in the oviduct, and histological and histochemical descriptions of the posterior oviduct in relation to sperm storage. The value of sperm storage in a fire-prone environment will also be discussed. I

hypothesise that female *B. pumilum* do store sperm in their oviduct, allowing for fertilisation in absence of a male in a post-fire environment.

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MATERIALS AND METHODS

Collection of Bradypodion pumilum

Female *Bradypodion pumilum* specimens were collected seasonally from Stellenbosch (3318DD) and Somerset West (3418BB), Western Cape, South Africa between February 2005 and January 2006 (Figure 2.2). The lizards were sacrificed within 24 hours of capture, fixed in 10 % formalin and preserved in 70 % ethanol. The specimens were deposited in the Ellerman Collection at Stellenbosch University, South Africa.

Assessment of specimens

The female reproductive tract was removed, dehydrated, cleared with Xylene, embedded in paraffin wax and sectioned at 5-7 µm. Reproductive tracts from five specimens were serially sectioned and stained with Ehrlich's Haematoxylin and Eosin. These slides were assessed for sperm storage structures. Sections of the posterior oviduct were prepared for 39 females in different reproductive stages and alternating slides were stained with Ehrlich's Haematoxylin and Eosin, PAS and Alcian blue 8GX (pH 1 and pH 2.8) (Humason 1979). These slides were assessed for presence and location of sperm and associated secretory substances. The same slides were also used to measure the height of ten epithelial cells for each specimen (Qwin software package, Leica). Sections of the gastro-intestinal tract and kidney were used as control slides. Two post-partum specimens (within 48 hours of delivery) were serially sectioned and stained with Ehrlich's Haematoxylin

and Eosin and assessed for presence of sperm in the oviduct. The two post-partum specimens were kept in solitary after parturition to ensure future copulation did not take place.

Statistical analysis

All statistics were performed using the SPSS (14th edition) software package. A value of $P < 0.05$ was considered significant. Data were assessed for a normal distribution. Standard error values are always stated.

A Kruskal Wallis Test and Dunn's post-hoc tests were used to see if any variation occurred between mean values of epithelial cell height, throughout the seasons of the year, the different reproductive stages and presence of sperm in the oviduct.

RESULTS

Bradypodion pumilum shows an aseasonal reproductive cycle thus all stages of the reproductive cycle are represented throughout the seasons of the year. Of the 46 females investigated 17 were pre-vitellogenic (two 48 hours post-partum), 14 were vitellogenic and 15 were gravid, 14 were collected in summer, nine in autumn, 11 in winter and 12 in spring.

Sperm presence

No evidence of specialised sperm storage structures were found in the serial sections of the reproductive tract of female *B. pumilum*. However, of the 46 sectioned females 73.9 % had sperm present in the posterior oviduct (Figure 4.1). More females had sperm present in the oviduct in winter than any other time of

year (Figure 4.2) and the greatest amount of sperm present occurred in winter (Figure 4.3). More females had sperm stored when they were reproductively active (Figure 4.4). Both post-partum females investigated had abundant sperm in the posterior oviduct (Figure 4.5).

General histology and secretory activity of the posterior oviduct

The posterior oviduct in *Bradypodion pumilum* is extremely folded with the mucosa consisting of ciliated simple columnar epithelial cells (Figure 4.6). Abundant melanophores were found in the submucosa and serosa layers. Secretory granules in the epithelial cells appeared in almost every cell. The epithelial cells in the posterior oviduct stained well with PAS and Alcian Blue 8GX pH 2.8 signifying the presence of carbohydrates and mucopolysaccharides (Figure 4.7). However, fewer cells stained well with Alcian Blue 8GX pH 1 signifying sulphated mucopolysachharides. The staining intensity of the epithelial cells of the posterior vagina with PAS did not change seasonally. However, more cells in the posterior oviduct stained with Alcian Blue 8GX pH 1 in spring and Alcian Blue 8GX pH 2.8 in autumn than in any other season. The posterior oviduct stained more intensely with PAS and Alcian Blue 8GX pH 1 in vitellogenic and gravid specimens than in pre-vitellogenic specimens. Alcian Blue 8GX pH 2.8 showed no variation throughout different stages of the reproductive cycle. The staining intensity of PAS, Alcian Blue 8GX pH 2.8 and Alcian Blue 8GX pH 1 in the posterior oviduct was always greater when sperm was present.

Epithelial cells of the posterior oviduct were significantly taller in winter than any other time of year (Kruskal Wallis; $H = 96.6$, $df = 3$, $P < 0.001$; Figure 4.8), in

vitellogenic females than females in any other reproductive phase (Kruskal Wallis; $H = 14.6$, $df = 2$, $P < 0.01$; Figure 4.9) and in females containing sperm than those without (Kruskal Wallis; $H = 18.4$, $df = 2$, $P < 0.001$; Figure 4.10).

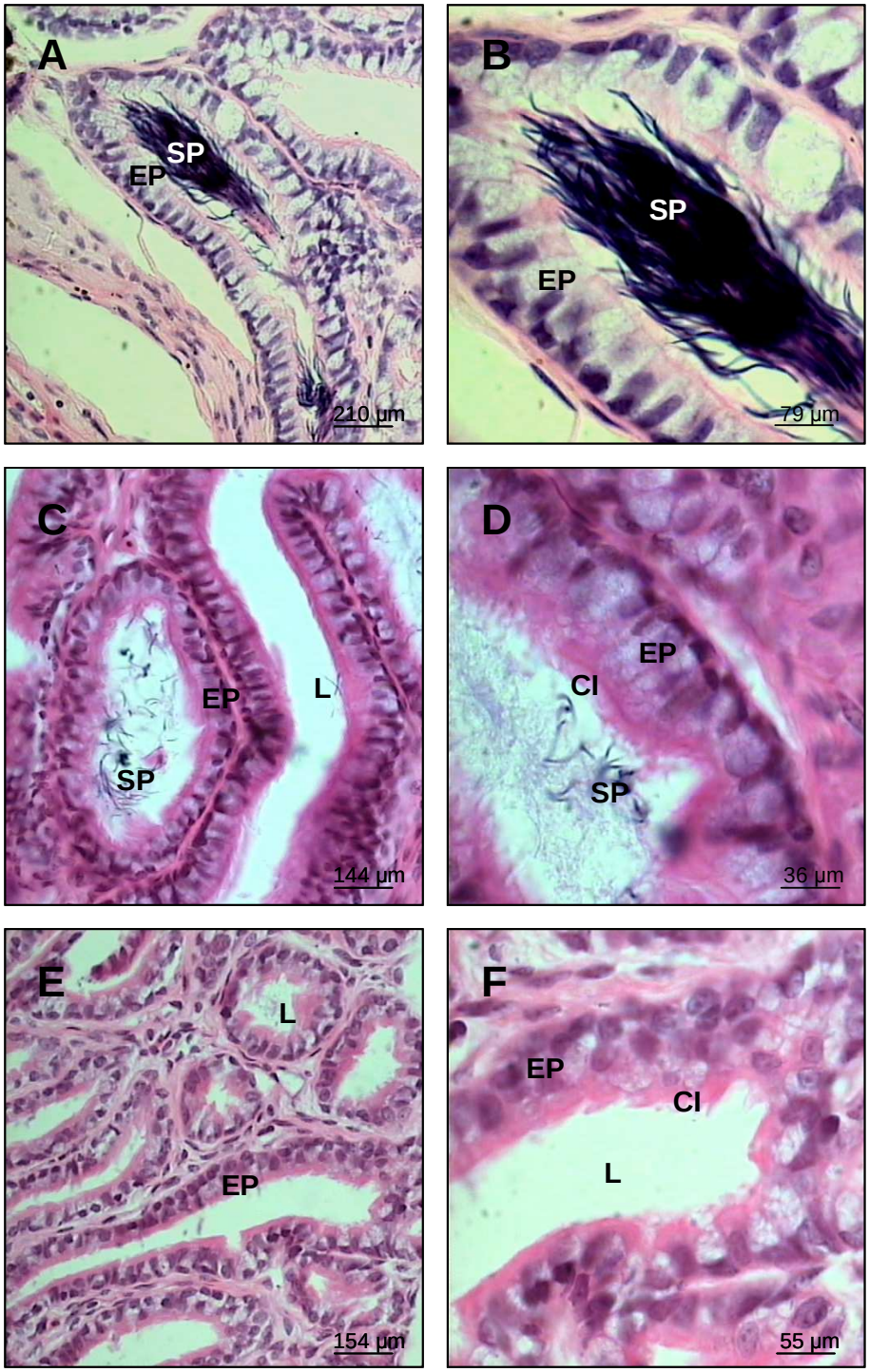


Figure 4.1 Photomicrographs of sections of the posterior oviduct of *Bradypodion pumilum* females stained with Ehrlich's Haematoxylin and Eosin. Gravid females with abundant sperm (A and B) and with few sperm (C and D) and a pre-vitellogenic female with no sperm present in the posterior oviduct (E and F) are indicated. EP = epithelial cells, SP = sperm, L = lumen and Cl = cilia.

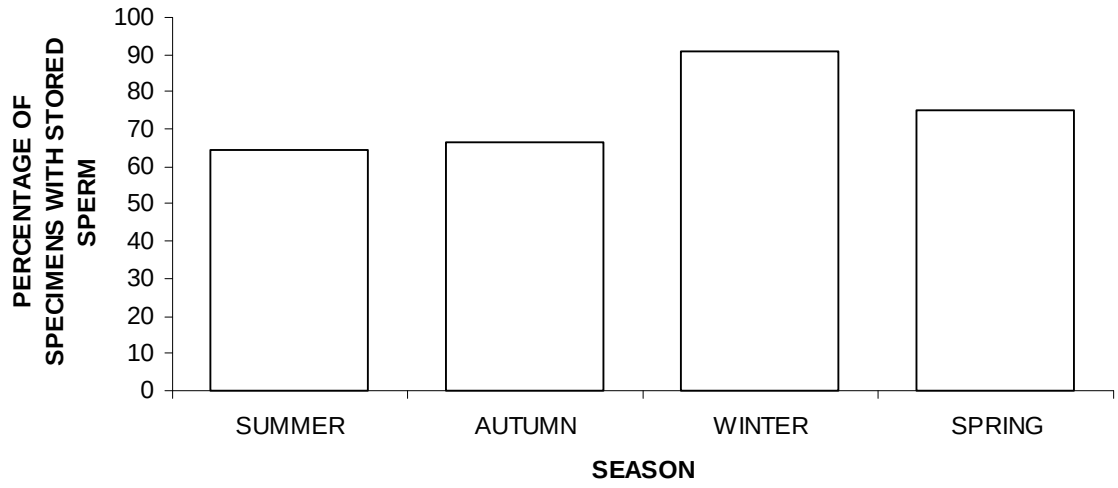


Figure 4.2 Column chart showing the percentage of *Bradypodion pumilum* females with sperm present in the posterior oviduct in the different seasons of the year.

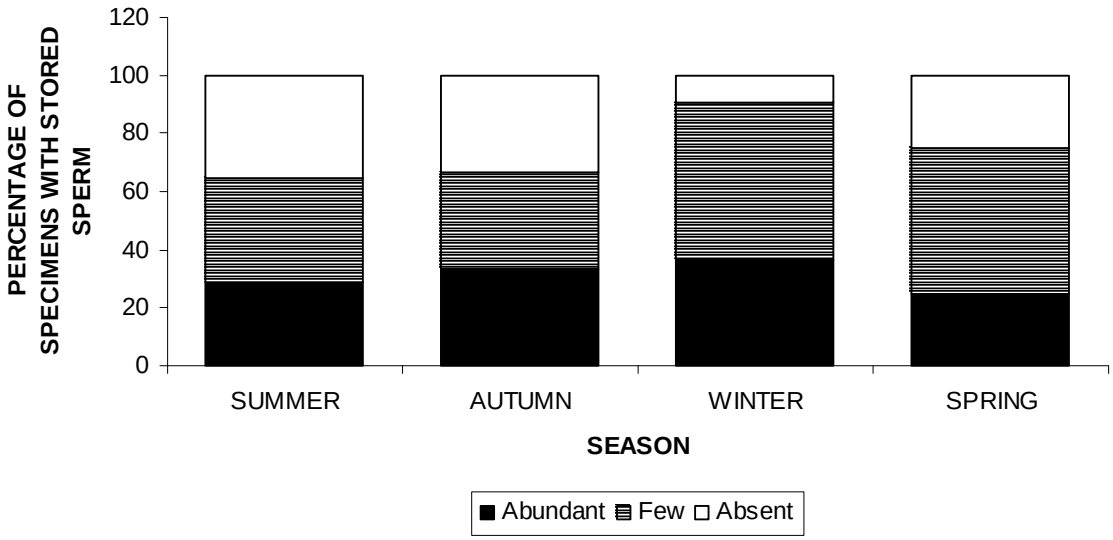


Figure 4.3 Column chart showing the percentage of *Bradypodion pumilum* females with different amounts of sperm in the posterior oviduct in the different seasons of the year.

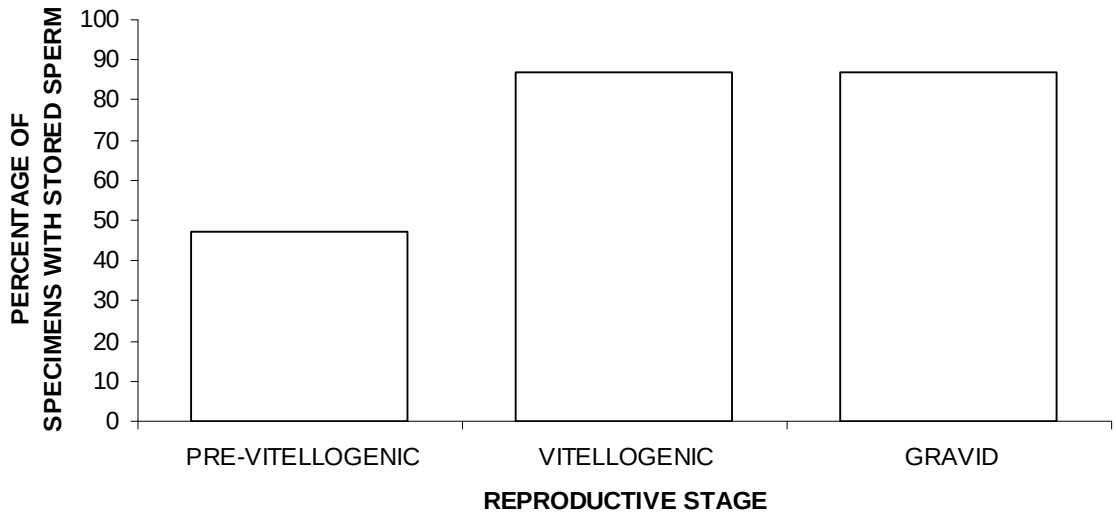


Figure 4.4 Column chart showing the percentage of *Bradypodion pumilum* females with stored sperm in the posterior oviduct at different stages of the reproductive cycle.

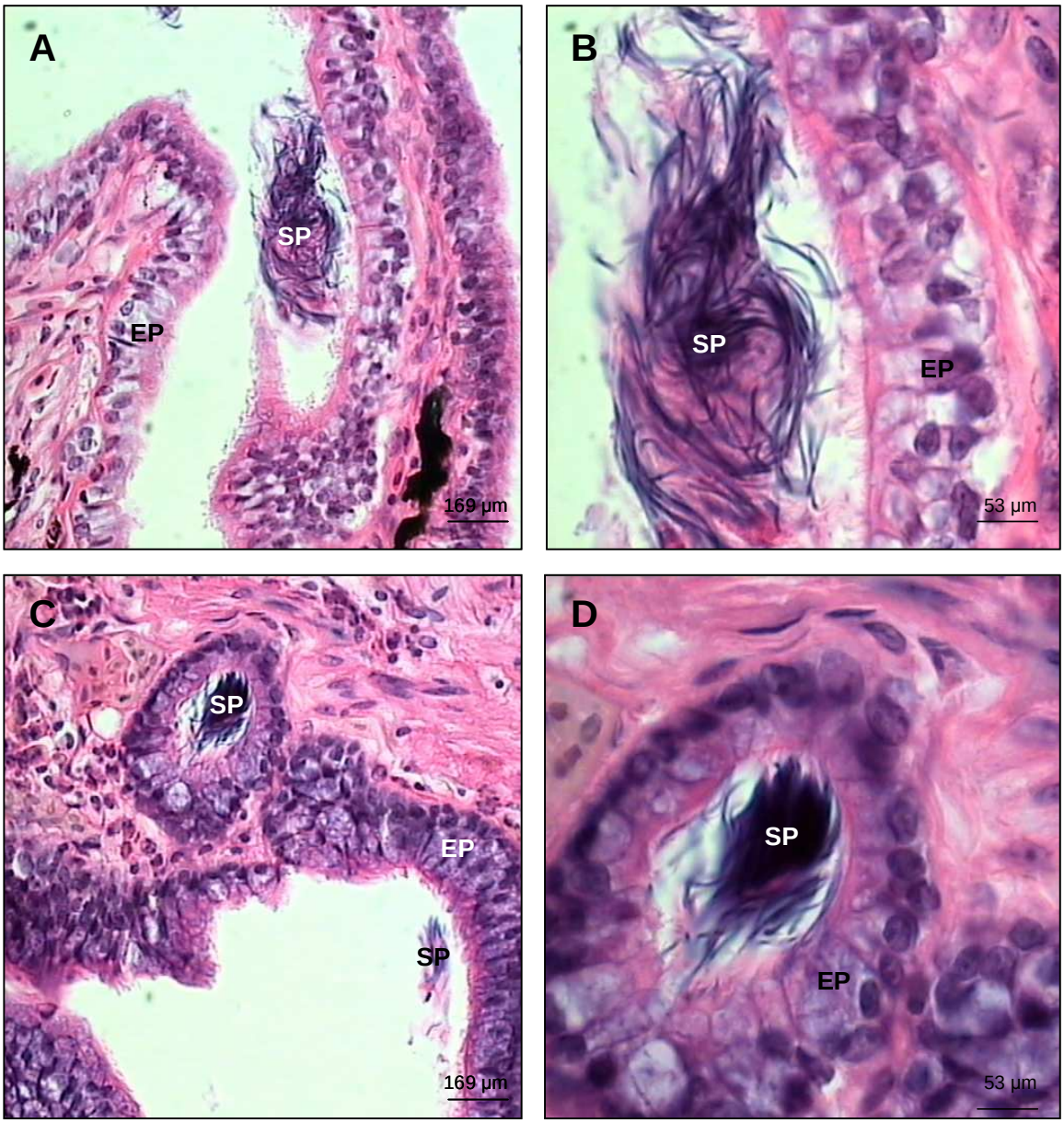


Figure 4.5 Photomicrographs of sections of the posterior oviduct of two post-partum females of *Bradypodion pumilum*, stained with Ehrlich's Haematoxylin and Eosin. Note abundant sperm in the posterior oviduct. EP = epithelium, SP = sperm.

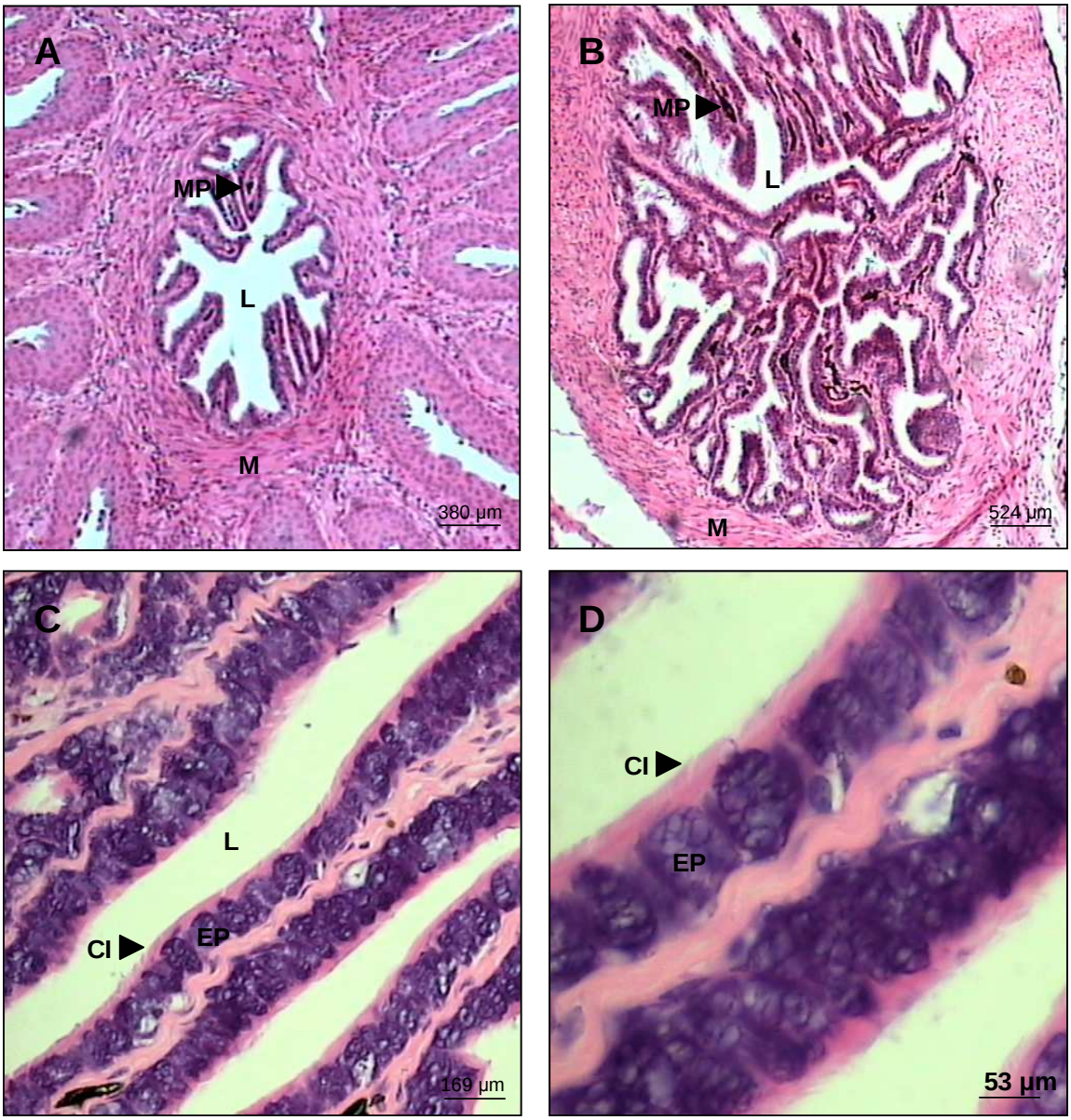


Figure 4.6 Photomicrographs of the posterior oviduct of female *Bradypodion pumilum* stained with Ehrlich's Haematoxylin and Eosin. A shows a very posterior section (x10), B shows an increase in folding on a more anterior section (x10), C shows a section of epithelial (x40) and D increased magnification (x100) of epithelial cells. A and B are of a late vitellogenic individual and C and D are of a gravid individual. EP = epithelium, SP = sperm, M = muscle, MP = melanophore, L = lumen and CI = cilia.

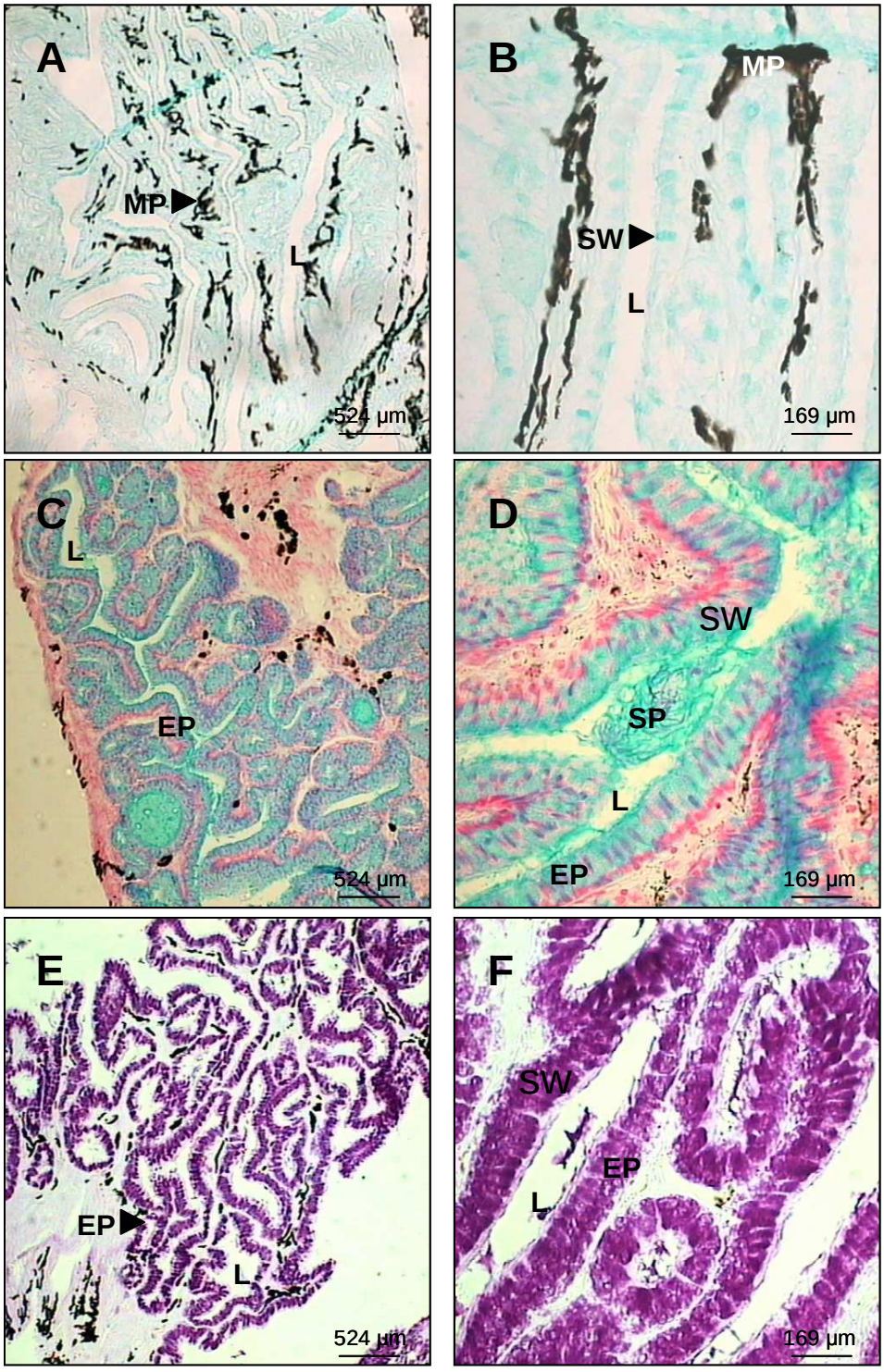


Figure 4.7 Photomicrographs of the posterior oviduct of female *Bradypodion pumilum* stained with Alcian Blue 8GX pH 1 (A and B), Alcian Blue 8GX pH 2.8 (C and D) and PAS (E and F). A, B and F are pre-vitellogenic females, C is an early vitellogenic female and D and E are gravid individuals. EP = epithelium, SP = sperm, MP = melanophore, L = lumen, SW = well stained cell and CI = cilia.

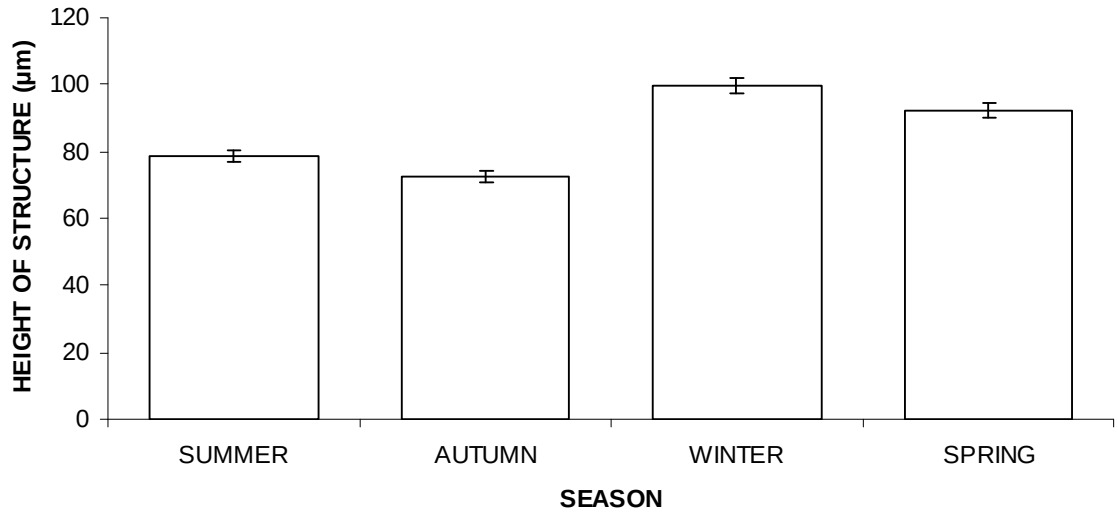


Figure 4.8 Column chart showing the mean height and standard error of the epithelial cells of the posterior oviduct of female *Bradypodion pumilum* during different seasons of the year. Dunn's post-hoc tests show that winter is significantly different to spring, summer and autumn, and spring is significantly different to summer and autumn ($P < 0.05$).

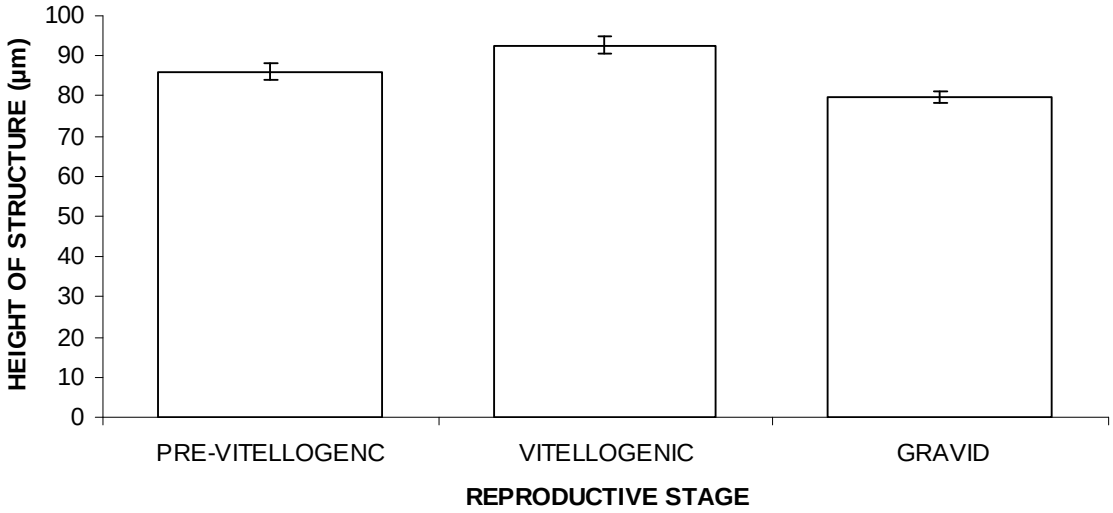


Figure 4.9 Column chart showing the mean height and standard error of the epithelial cells of the posterior oviduct of female *Bradypodion pumilum* during different stages of the reproductive cycle. Dunn's post-hoc tests show that vitellogenic females are significantly different to both gravid and pre-vitellogenic females ($P < 0.05$).

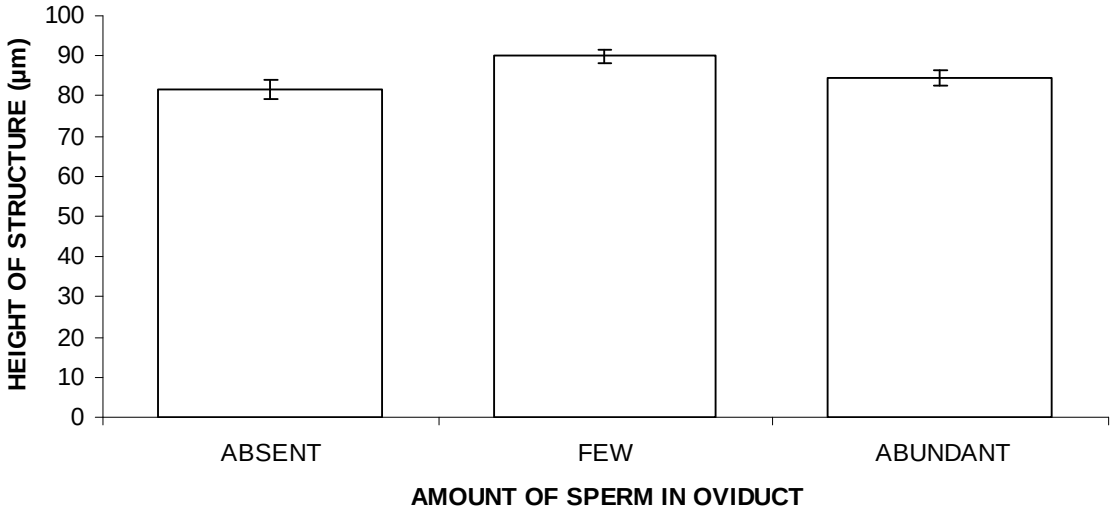


Figure 4.10 Column chart showing the mean height and standard error of the epithelial cells of the posterior oviduct of female *Bradypodion pumilum* with absent, few or abundant sperm in the posterior oviduct. Dunn's post-hoc tests show that females with few sperm in their posterior oviduct are significantly different to those with sperm absent ($P < 0.05$).

DISCUSSION

Sperm presence or sperm storage?

Sperm storage tubules are generally considered invaginations of the oviduct epithelium with less ciliated cells at the tip of the tubules (Gist and Jones 1987). These specialised sperm storage receptacles have been found in lizards before (see Sever and Hamlett 2002) even in chameleons (Saint Girons 1962), but I found *Bradypodion pumilum* females did not contain any specialised sperm storage structures. However, the findings in this chapter suggest that specialised structures are not always necessary for sperm storage to occur. The presence of sperm in most *B. pumilum* oviducts throughout the year and reproductive stages suggests that sperm is indeed being stored rather than evidence of a recent copulation. Further support is the occurrence of sperm in individual females after the delivery of a clutch and before a new copulation. Whilst some reptiles do contain sperm storage tubules, other reptiles show sperm storage without specialised structures and some of them store sperm for prolonged periods (see Sever and Hamlett 2002 for a review). Conversely, some cordylid species have specialised sperm storage structures even when they appear unnecessary due to their synchronised male and female reproductive cycles (Flemming 2006). The definition of what the term ‘sperm storage' includes, needs more attention in the scientific literature.

Sperm storage in female **Bradypodion pumilum**

Bradypodion pumilum females have more sperm present in autumn and winter, and taller epithelial cells, in the posterior oviduct in winter than females at any other time of year. An increase in mucopolysaccharides also occurs at this time. It

is possible that a peak in mating activity occurs around autumn/winter. Males show a peak in spermiogenic activity, Leydig cell size and they store the most sperm in autumn, but then a slight regression of the testis occurs and the amount of stored sperm decreases (Chapter 3). It is around this time that the number of females with stored sperm increases.

When female *B. pumilum* are reproductively active (either vitellogenic or gravid) and sperm is present the epithelial cells are taller and there are more secretions in the posterior oviduct (carbohydrates and sulphated mucopolysaccharides). This increased secretory activity and secretions may help to nourish sperm present in the oviduct.

Bradypodion pumilum females are more likely to store sperm when they are reproductively active. The decreased amount of sperm stored in non-reproductive (pre-vitellogenic) individuals suggests that sperm may not always be stored over reproductive seasons or for prolonged periods. It is possible due to the lack of sperm storage tubules that sperm present in the oviduct may be carried out with the young during birth. However, two females sectioned post-partum contained a large amount of sperm in the posterior oviduct. Due to the highly folded nature of the oviduct wall it is possible that some sperm may be retained in these folds during the passing of young.

The hypothesis suggested by Cohen (1977) that sperm storage occurs to synchronise male and female reproductive cycles, seems unlikely as the main reason for sperm storage in this species. *Bradypodion pumilum* shows an

aseasonal reproductive cycle, although the males show two peaks in spermatogenic activity, sperm is available and males appear ready to mate the entire year round. Hence, reproductively active specimens are found throughout the year and so sperm storage is not necessary to synchronise male and female cycles.

The cryptic female choice hypothesis (Birkhead *et al.* 1993; Eberhard 1998) by way of sperm competition or mate choice appears a likely candidate for sperm storage in *B. pumilum*. Females may theoretically 'choose' which sperm will fertilise her eggs possibly by a chemical cue in the males' semen. Alternatively, females may mate with many males (males appear ready year round) and their sperm may be grouped in the posterior oviduct to encourage sperm competition so that only the best males and hence the best sperm fertilise the female's ova. Sperm competition could lead to multiple paternity with the best male siring the majority if not all of the female's ova. It is unknown at present if *B. pumilum* shows multiple paternity.

It is likely that female *B. pumilum* produce several clutches per year (Chapter 2). However, storing sperm to minimise copulation frequency (Conner and Crews 1980) seems an unlikely explanation as the main reason for sperm storage in this species. However, for the fertilisation of further clutches hypothesis (Conner and Crews 1980) it appears possible for *B. pumilum*, at least in captivity, to reproduce from sperm stored in the oviduct (Atsatt 1953) but due to the low incidence of sperm in non-reproductive females it seems unlikely that sperm is stored for

prolonged periods. However, again the two post-partum females studied contained a large amount of sperm in their posterior oviduct.

Bradypodion pumilum is known to inhabit areas that are fire-driven and whilst prolonged periods of sperm storage may be unnecessary, a production of a further clutch without a male would be beneficial. Arboreal lizards are known to be isolated for significant periods in small patches of vegetation after a fire (Du Toit 2001). If a female is isolated in a patch of vegetation after a fire she may be able to reproduce in absence of a male and hence start to re-populate the area as the vegetation recovers. Anecdotal observations of females (in a laboratory environment) suggest that at least two clutches may be produced after a female is isolated from males (pers. obs.).

CONCLUSION

Female *B. pumilum* do not have specialised sperm storage structures, they store sperm in the lumen of the highly folded posterior oviduct. Sperm storage and associated oviductal changes show some seasonality, some relation to the reproductive stage of the individual female, and some relation to the male cycle. Due to the aseasonality of both male and female reproductive cycles, cryptic female choice may explain female sperm storage in *B. pumilum.* However, sperm storage may also be beneficial to isolated females enabling them to reproduce without a male allowing for speedy re-population of an area after a fire. Although the unusual reproductive strategy of *B. pumilum* appears to be an adaptation to life in an unpredictable fire-prone environment, to establish this, the reproductive

strategy of species closely related to *B. pumilum* that do not inhabit fire-prone areas must be studied.

CHAPTER FIVE

THE REPRODUCTIVE STRATEGY OF *BRADYPODION*

INTRODUCTION

Reproduction in chameleons

Chameleon reproductive cycles have received little attention in the literature and previous studies have focused on factors specifically affecting the female reproductive cycle (Menzies 1958; Cuadrado and Loman 1999; Diaz-Paniagua *et al*. 2002) but few concentrate on the male testicular cycle, its relation to the female cycle and how it is affected by environmental variables.

Lin and Nelson (1981) studied the reproductive cycles of both male and female *Chamaeleo jacksonii* and *C. hoehnelii*, two chameleon species inhabiting the same aseasonal tropical area. Both species belong to a lineage of viviparous chameleons from East Africa. These two chameleons experience the same abiotic conditions but biotic factors have shaped their reproductive strategies differently. *Chamaeleo jacksonii* is a seasonal reproducer whilst *C. hoehnelii* reproduces aseasonally. The authors suggest this difference in reproductive strategy is due to a higher predation pressure on *C. hoehnelii*.

Dwarf chameleons (Bradypodion)

Bradypodion are small, viviparous, insectivorous chameleons endemic to South Africa, Lesotho and Swaziland (Figure 1.1) occurring in a variety of vegetation types and climatic conditions (Figure 1.2). Dwarf chameleons (*Bradypodion*) have a separate origin of viviparity than the aforementioned East African viviparous ones (Blackburn 1999). There are currently 15 recognised species (Branch 1998; Branch *et al*. 2006; Tolley and Burger 2007) with the possibility of a number of new species being described given the number of unique genetic lineages found

(Tolley *et al.* 2004; Tolley and Burger 2007). The reproductive strategies of *Bradypodion* have received little attention in the scientific literature. Langewerf (1992) made anecdotal observations on the reproductive cycle of *B. thamnobates* based on a relocated, captive population in America. He concluded that female *B. thamnobates* give birth in March, April and May. However, this may not be an accurate assessment of the reproductive cycle of female *B. thamnobates* as captive reptile reproduction may be atypical (Blackburn *et al.* 2003).

The Cape Dwarf Chameleon, *Bradypodion pumilum* is the only *Bradypodion* species for which detailed reproductive data exist. Female *B. pumilum* show an aseasonal reproductive cycle, with high clutch sizes and it is likely that individuals produce multiple clutches each year (Chapter 2). Male *B. pumilum* show elements of a bimodal reproductive cycle with peaks in spermatogenic activity in autumn and spring/summer, although the testes never fully regress (Chapter 3). Male and female *B. pumilum* are known to store sperm (Chapter 3 and 4). It is thought that this unusual reproductive strategy may help to ensure species survival in an unpredictable fire-prone habitat.

AIM

Bradypodion pumilum show unusual reproductive characteristics for a temperate-zone, viviparous lizard with female aseasonality, high fecundity, a prolonged male cycle and the ability to store sperm in both sexes. It has been suggested that this is an adaptation to life in an unpredictable fire-prone habitat (Chapters 2 and 3). Other species within the *Bradypodion* genus do not inhabit unpredictable fire-driven environments. It is therefore necessary to investigate the reproductive

strategy of other *Bradypodion* species to establish whether *B. pumilum* has a reproductive adaptation to unpredictable environments. I hypothesise that, as in the East African viviparous chameleons, reproduction in dwarf chameleons may be varied within the genus due to environmental conditions. If reproduction is seasonal in members of *Bradypodion* that do not inhabit fire-prone areas then *B. pumilum* exhibits a reproductive adaptation to life in an unpredictable environment. Therefore this study aims to conduct the first detailed investigation of the reproductive parameters of species within the genus *Bradypodion*, focussing specifically on fecundity, seasonality and synchronicity amongst individual females, and the spermatogenic cycle amongst individual males.

MATERIALS AND METHODS

Specimen collection

Bradypodion specimens from collections in the Natal Museum, Port Elizabeth Museum, Transvaal Museum and the Ellerman Collection at Stellenbosch University were inspected.

Bradypodion pumilum (results taken from Chapter 2, 3 and 4), *B. transvaalense*, *B. ventrale*, *B. occidentale, B. melanocephalum* and male specimens of *B. dracomontanum* were selected to be studied in greater detail than those of other *Bradypodion* species for which samples were available. The selected species were chosen as they represent all of the well supported clades in the current *Bradypodion* phylogeny (Tolley *et al.* 2004; Tolley *et al*. 2006; Figure 1.3), they are distributed throughout southern Africa in a variety of vegetation types and climate regimes (Figures 1.1 and 1.2), and sufficient samples were available for these

species. Samples were also available for female *B. dracomontanum*, *B. taeniabronchum*, *B. thamnobates*, *B. damaranum*, *B setaroi*, and *B. gutturale* and male *B. thamnobates*, *B. nemorale* and *B. gutturale*. However, due to the lack of frequent, sufficient samples only anecdotal observations were made on these species.

Assessment of specimens

To assess the female reproductive cycle both ovaries were examined and reproductive status was categorised according to the methods of Van Wyk and Mouton (1998); by grouping individual females into four categories based on the appearance of follicles and developing embryos. The four categories were pre-vitellogenic (for translucent, unyolked follicles of 2 mm in diameter or less), early vitellogenic (for yolked follicles between 2 mm and 5 mm in diameter), late vitellogenic (for yolked follicles 5-7 mm in diameter), and gravid. Oviductal eggs were counted for the evaluation of clutch size and SVL (mm) was recorded.

To assess the male reproductive cycle the left testis was removed and the longest and shortest diameter of the testis was measured to the nearest 0.01 mm using digital callipers. These measurements were used to calculate testicular volume using the formula for an ellipsoid; $V = 4/3\ \pi\ a^2\ b$ where V equals volume, *a* is half the shortest diameter and *b* is half the longest diameter (Selby 1965). The testes were dehydrated, cleared with Xylene, embedded in paraffin wax and sectioned at 5-7 µm. Sections were stained using Ehrlich's Haematoxylin and Eosin and digital photographs of slides were taken (QWin software package, Leica). Diameters of ten seminiferous tubules were measured for each specimen and seasonal

histological changes in the testis were recorded qualitatively using the methods of (Licht (1967ab), Mayhew (1971) and Van Wyk (1995).

Statistical analysis

All statistics were performed using the SPSS (14th edition) software package. A value of $P < 0.05$ was considered significant. Data were assessed for a normal distribution and transformed where appropriate. Standard error values are always stated.

Female dwarf chameleons - Spearman's Rank Order Correlation analysis was performed to test for the effect of SVL on clutch size in *B. pumilum*. Pearson's Correlation Coefficient analysis was used to investigate the effect of SVL on clutch size in *B. transvaalense*, *B. ventrale*, *B. occidentale* and *B. melanocephalum*. Analysis of Covariance, with SVL as covariate, was used to test for monthly variations in clutch size for *B. transvaalense and B. pumilum*. Analysis of Variance was used to test for monthly variations in clutch size for *B. occidentale* and *B. ventrale*. A Kruskal-Wallis test was used to test for monthly variations in clutch size for *B. melanocephalum.* Mean, range and standard errors were calculated for clutch size and SVL for *B. damaranum*, *B. dracomontanum*, *B. taeniabronchum*, *B. thamnobates*, *B setaroi* and *B. gutturale*. The percentage of gravid females present throughout the year was also calculated.

Male dwarf chameleons - To establish the relationship between testis volume and male SVL in *B. transvaalense* and *B. pumilum*, a Spearman's Rank Order Correlation was used. Pearson's Correlation Coefficients were used to establish

the relationship between testis volume and SVL in *B. melanocephalum*, *B. ventrale*, *B. occidentale* and *B. dracomontanum*. Analysis of Covariance with Bonferroni post-hoc tests were used to establish if mean monthly values of testis volume varied in *B. transvaalense* and *B. pumilum*. Analysis of Variance and Tukey's post-hoc tests were used to establish the variation in mean monthly values of testis volume in *B. melanocephalum*, *B. ventrale*, *B. occidentale* and *B. dracomontanum*. Analysis of Variance with Tukeys post-hoc comparisons were used to test for significant differences in mean monthly values of seminiferous tubule diameters in *B. pumilum*, *B. transvaalense*, *B. melanocephalum*, *B. ventrale* and *B. dracomontanum*. A Kruskal-Wallis test with Dunn's post hoc tests was used to test for mean monthly differences in the seminiferous tubule values of *B. occidentale*.

RESULTS

Considerable debate occurs concerning species classifications within the genus *Bradypodion*. However, for the purpose of this study, only the museum specimens occurring within the boundaries of the current species distributions (Branch 1998) were used. The only exception is *B. karrooicum*. It is generally thought that *B. karrooicum* is the same species as *B. ventrale* (Branch 1998; Branch *et al.* 2006) and hence all specimens labelled *B. karrooicum* were included in *B. ventrale* for this analysis.

Female dwarf chameleon reproduction

Sexual maturity was determined at 48 mm for *B. transvaalense*. Due to small sample sizes for other species of dwarf chameleon, a general size at sexual

maturity was determined for the small bodied (42 mm) and the large bodied dwarf chameleons (46 mm) as small and large bodied are defined in Branch (1998) (Table 5.1). The percentage of gravid females present for all species of dwarf chameleon is consistently high. Clutch sizes appear varied but are generally high in all species (Table 5.1).

Female *B. pumilum* (Figure 5.1A), *B. transvaalense* (Figure 5.1B), *B. ventrale* (Figure 5.2A), *B. occidentale* (Figure 5.2B) and *B. melanocephalum* (Figure 5.3) do not exhibit a distinct seasonal pattern of reproductive activity and hence individual females are not completely synchronised.

Clutch size showed a significant positive relationship with female SVL in *B. pumilum* (Spearman's Rank Order Correlation; $r = 0.500$, $P < 0.01$; Figure 5.4A) *B. transvaalense* (Pearson's Correlation Coefficient; $r = 0.511$, $P < 0.01$; Figure 5.4B) and *B. melanocephalum* (Pearson's Correlation Coefficient; $r = 0.821$, $P < 0.05$; Figure 5.5). *Bradypodion ventrale* (Pearson's Correlation Coefficient; $r = 0.046$, $P > 0.05$) and *B. occidentale* (Pearson's Correlation Coefficient; $r = 0.545$, $P > 0.05$) did not show any significant relationship between clutch size and SVL. Mean monthly clutch size does not vary throughout the year for *B. pumilum* (ANCOVA; $F = 0.978$, $P > 0.05$), *B. occidentale* (ANOVA; $F = 5.11$, $P > 0.05$), *B. ventrale* (ANOVA; $F = 0.385$, $P > 0.05$) or *B. melanocephalum* (Kruskal-Wallis Test; $H = 3.53$, $df = 3$, $P > 0.05$). *Bradypodion transvaalense* does show a significant monthly variation in clutch size (ANCOVA; $F = 3.38$, $P < 0.05$; Figure 5.6).

No definitive conclusions can be made from the female samples available for the other *Bradypodion* species (Table 5.2) but six individuals (highlighted in red) are females that are atypical of seasonal summer breeding. However, this is not an accurate representation of the reproductive cycle of these species due to the shortage of collections during the winter period.

Male dwarf chameleon reproduction

Sexual maturity was determined at 52 mm for large bodied species (*B. transvaalense*, *B. ventrale*, *B. occidentale* and *B. dracomontanum*) and 48 mm for small bodied (*B. melanocephalum*). Sexual maturity was determined at 42 mm for *B. pumilum* (see Chapter 3).

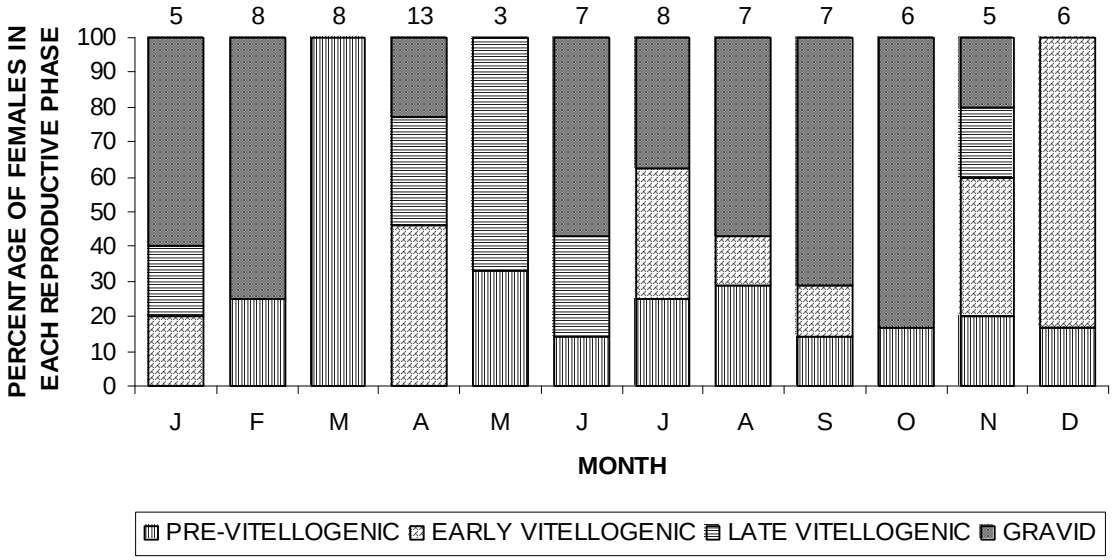
Snout-vent length has a significant positive relationship with testis volume in *B. transvaalense* (Spearman's Rank Order Correlation; $r = 0.632$, $P < 0.01$) and *B. pumilum* (Spearman's Rank Order Correlation; $r = 0.642$, $P < 0.001$) but not in *B. melanocephalum* (Pearson's Correlation Coefficient; $r = 0.544$, $P > 0.05$), *B. ventrale* (Pearson's Correlation Coefficient; $r = 0.295$, $P > 0.05$), *B. occidentale* (Pearson's Correlation Coefficient; $r = 0.510$, $P > 0.05$) and *B. dracomontanum* (Pearson's Correlation Coefficient; $r = 0.396$, $P > 0.05$). Mean testis volume values varied significantly throughout the year in *B. pumilum* (ANCOVA; $F = 3.82$; $P < 0.001$; Figure 5.7A) and *B. ventrale* (ANOVA; $F = 5.13$, $P < 0.05$; Figure 5.7B) but not in *B. transvaalense* (ANCOVA; $F = 0.269$, $P > 0.05$; Figure 5.7A), *B. melanocephalum* (ANOVA; $F = 1.52$, $P > 0.05$; Figure 5.7B), *B. occidentale* (ANOVA; $F = 3.95$, $P > 0.05$; Figure 5.8) and *B. dracomontanum* (ANOVA; $F = 5.51$, $P > 0.05$; Figure 5.8).

Mean monthly seminiferous tubule diameters varied significantly throughout the year in *B. pumilum* (ANOVA; $F = 12.8$, $P < 0.001$; Figure 5.9A), *B. transvaalense* (ANOVA; $F = 14.1$, $P < 0.001$; Figure 5.9A), *B. ventrale* (ANOVA; $F = 29.6$, $P < 0.001$; Figure 5.9B), *B. melanocephalum* (ANOVA; $F = 21.7$, $P < 0.001$; Figure 5.9B), *B. occidentale* (Kruskal-Wallis Test; $H = 37.7$, $df = 3$, $P < 0.001$; Figure 5.10) and *B. dracomontanum* (ANOVA; $F = 20.5$, $P < 0.001$; Figure 5.10).

Sperm was available throughout the year for male *B. pumilum* (Table 5.3A), *B. transvaalense* (Table 5.3B), *B. ventrale* (Table 5.4A), *B. melanocephalum* (Table 5.4B), *B. occidentale* (Table 5.5A) and *B. dracomontanum* (Table 5.5B). *Bradypodion gutturale* had males at spermatogenic stage five and seven in October, *B. nemorale* and *B. thamnobates* had males at spermatogenic stage six in March.

Table 5.1 Table showing the number of female specimens available (*N*); whether the species are small (S) or large (L) bodied as defined in Branch (1998); mean (mm), range (mm) and standard error of body size (SVL); size at sexual maturity (mm); mean, range and standard error of clutch size and the number of gravid females (*N*); and the percentage of gravid females throughout the year, for each species.

SPECIES	*N*	S/L	SVL	SVL RANGE	SVL MATURITY	CLUTCH SIZE	CLUTCH SIZE RANGE (*N*)	PERCENTAGE GRAVID
B. pumilum	88	L	69.4 ± 1.1	48.1 – 97.6	53	8.3 ± 0.6	3 – 20 (32)	39.8
B. transvaalense	96	L	62.0 ± 1.3	33.0 – 84.5	48	9.6 ± 0.7	3 – 16 (30)	38.8
B. ventrale	44	L	70.9 ± 1.3	53.8 – 88.5	46	10.3 ± 0.3	9 – 12 (9)	40.5
B. occidentale	26	L	74.7 ± 2.2	56.0 – 93.0	46	10.5 ± 1.3	6 – 16 (8)	41.7
B. melanocephalum	19	S	51.1 ± 1.7	34.0 – 63.5	42	9.0 ± 1.9	5 – 16 (5)	29.4
B. damaranum	13	L	50.4 ± 3.7	36.0 – 77.5	46	-	-	-
B. dracomontanum	7	L	55.4 ± 4.2	34.5 – 68.5	46	5.0	5 (1)	16.7
B. gutturale	5	L	75.3 ± 6.3	57.0 – 95.0	46	12.5 ± 1.5	11 – 14 (2)	40.0
B. setaroi	12	S	46.7 ± 3.2	33.0 – 64.0	42	8.0 ± 1.7	5 – 11 (3)	44.4
B. taeniabonchum	4	S	72.7 ± 2.6	69.0 – 80.3	42	7.8 ± 1.0	5 – 10 (4)	100.0
B. thamnobates	11	L	62.5 ± 2.6	46.5 – 76.0	46	7.7 ± 1.3	5 – 9 (3)	27.3



B

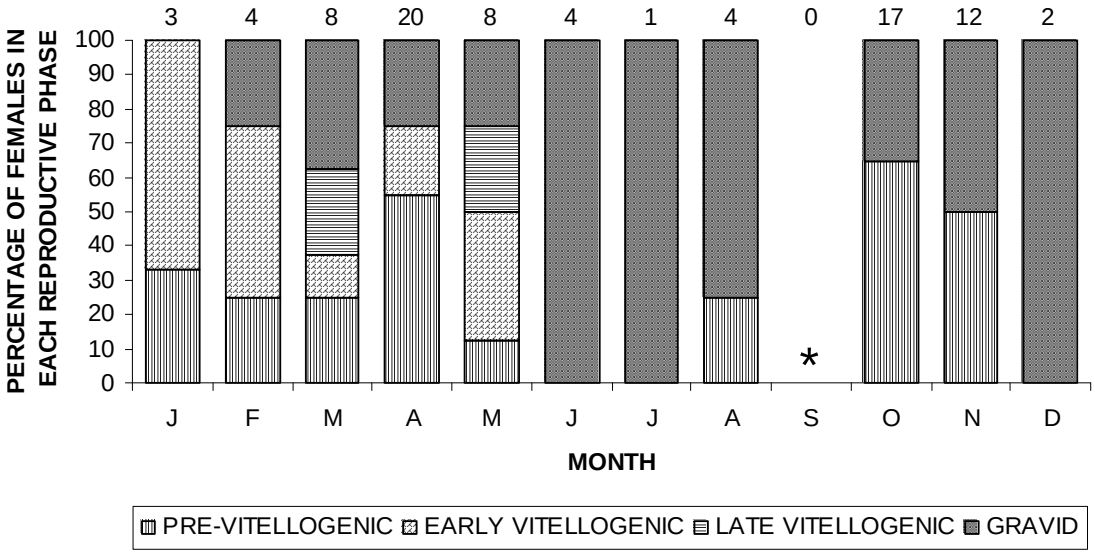
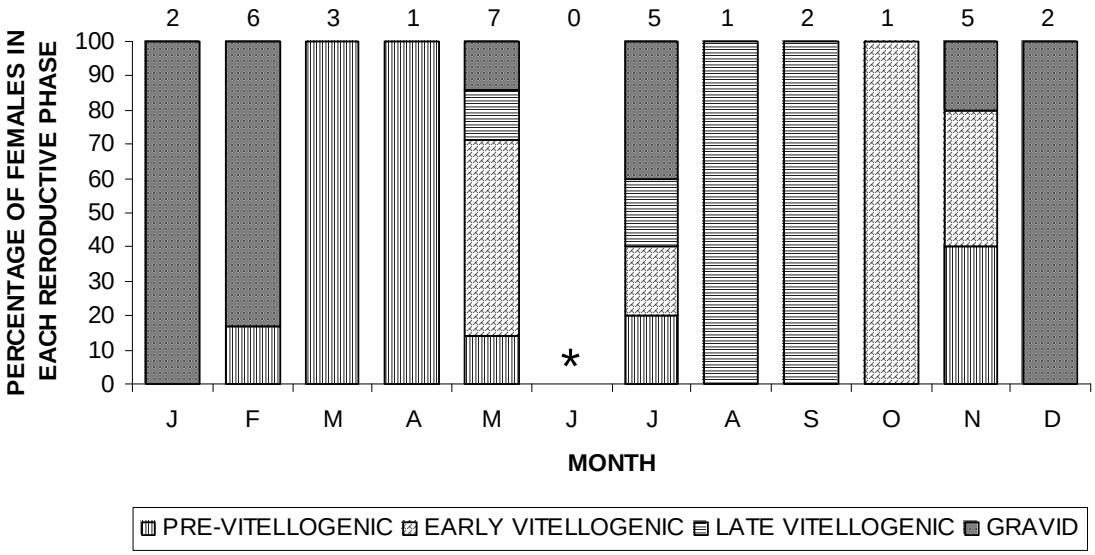


Figure 5.1 Column chart illustrating the reproductive phases of *Bradypodion pumilum* (A) and *B. transvaalense* (B) throughout the year. Monthly sample sizes are shown above columns. The asterisks denote months where specimens were not available.

A



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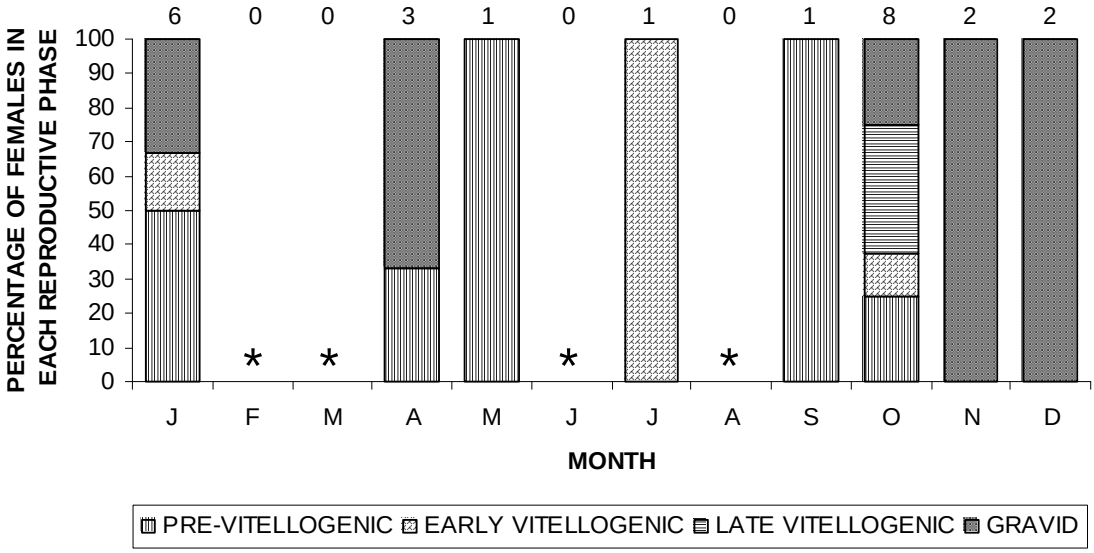


Figure 5.2 Column chart illustrating the reproductive phases of *Bradypodion ventrale* (A) and *B. occidentale* (B) throughout the year. Monthly sample sizes are shown above columns. The asterisks denote months where specimens were not available.

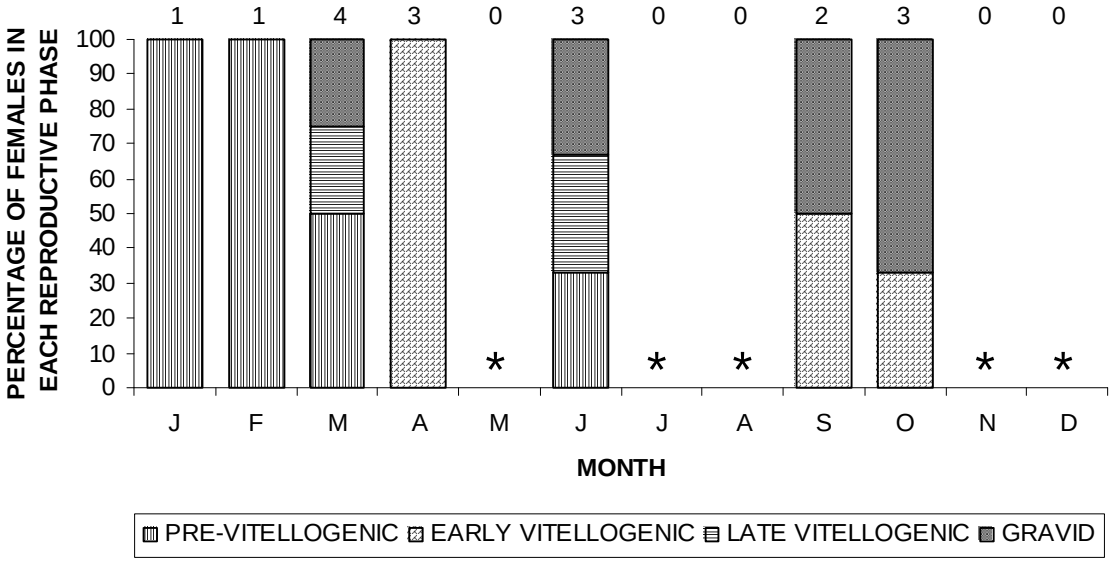
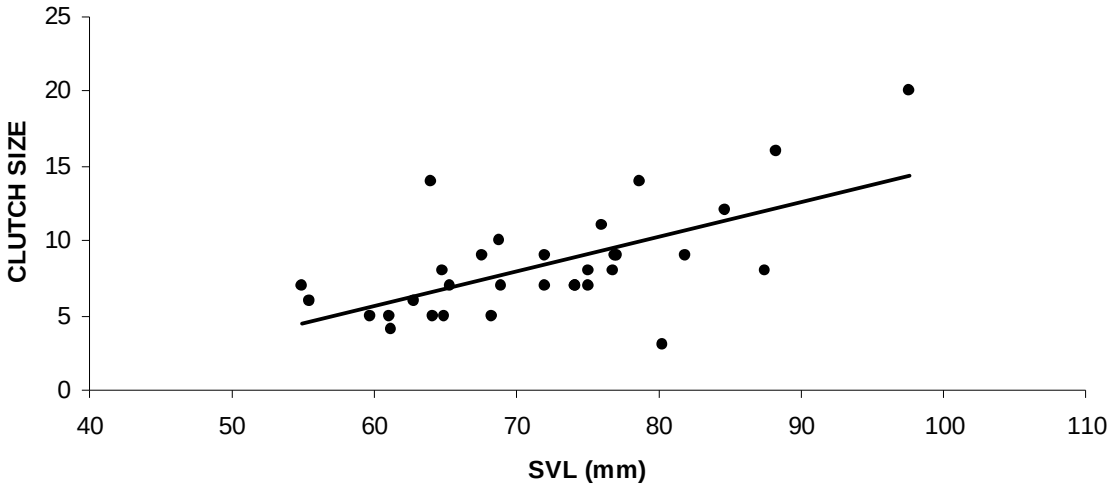


Figure 5.3 Column chart illustrating the reproductive phases of *Bradypodion melanocephalum* throughout the year. Monthly sample sizes are shown above columns. The asterisks denote months where specimens were not available.

A



B

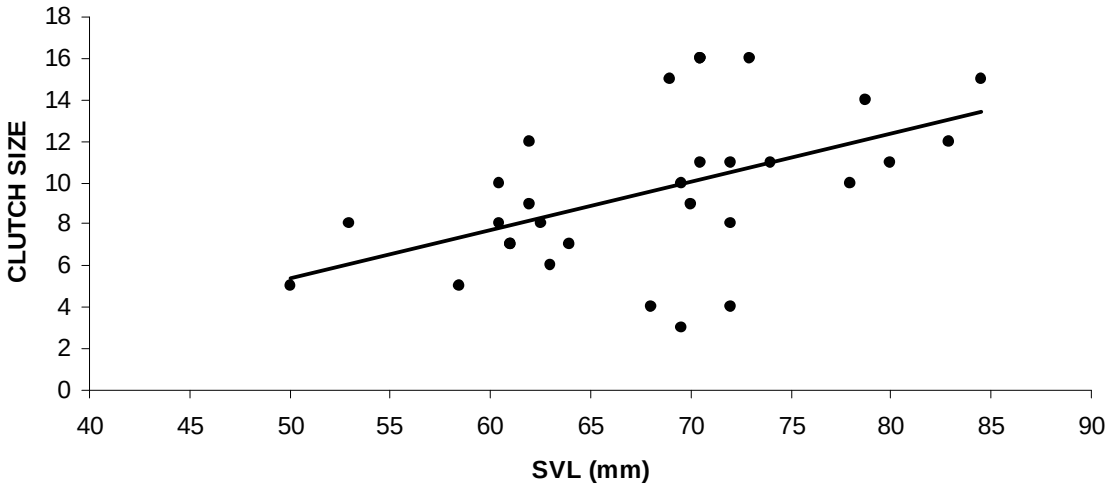


Figure 5.4 Scatterplot showing the relationship between female SVL and clutch size of *B. pumilum* (A) and *B. transvaalense* (B).

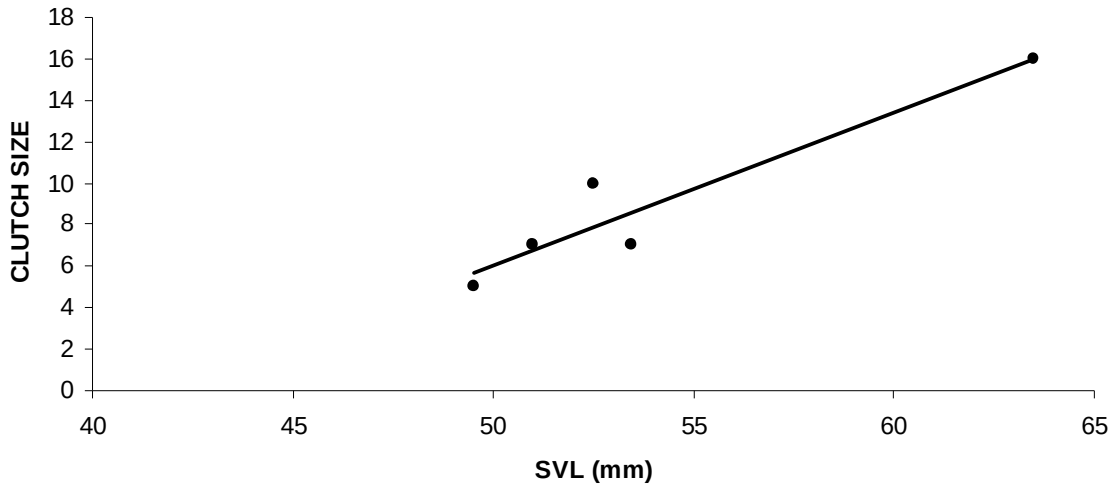


Figure 5.5 Scatterplot showing the relationship between female SVL and clutch size of *B. melanocephalum*.

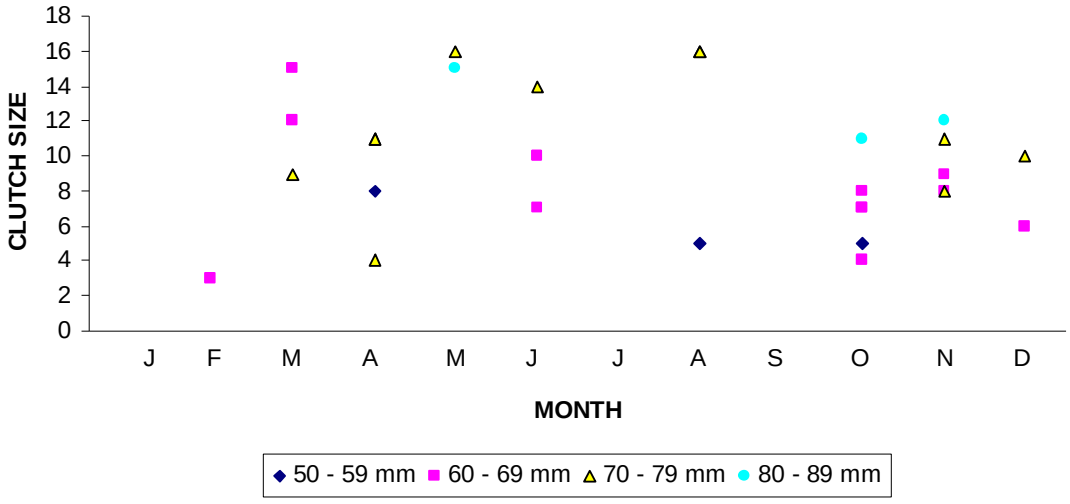
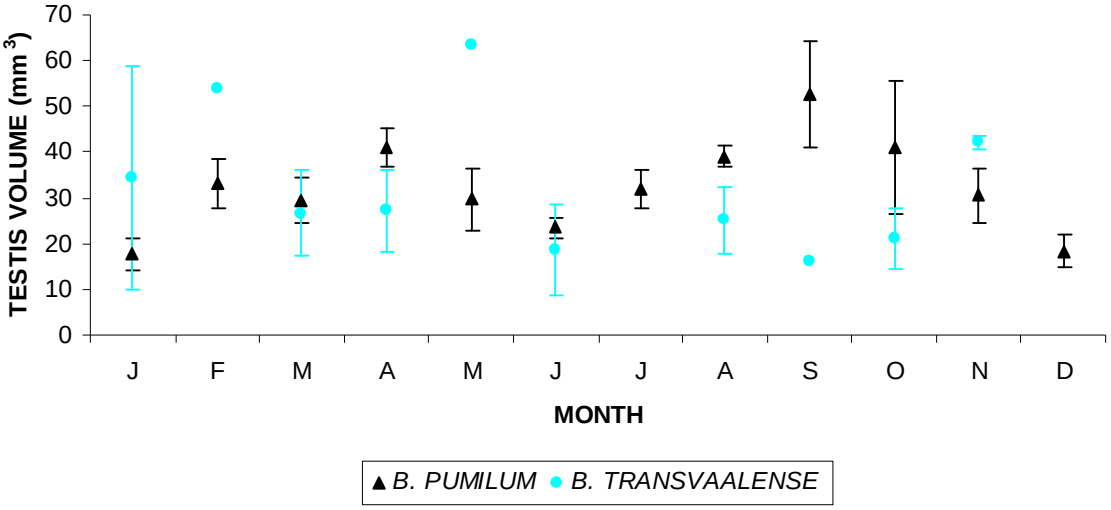


Figure 5.6 Graph showing the clutch size variation throughout the year for different size classes (mm) of *Bradypodion transvaalense*. Post-hoc tests showed no significant differences among months.

Table 5.2 Table showing the different reproductive stages of different *Bradypodion* species throughout the months of the year. Each symbol represents an individual female. Pre-vitellogenic = ▲, early vitellogenic = ■, late vitellogenic = ●, gravid = Δ. Red indicates a female that lies outside of the normal seasonal cycle.

SPECIES	J	F	M	A	M	J	J	A	S	O	N	D
B. damaranum		▲■■■			▲■					▲	▲▲▲	
B. dracomontanum	▲		▲	●							▲Δ	▲
B. gutturale			▲							■■ΔΔ		
B. setaroi	Δ		▲Δ	▲	▲					▲		▲ΔΔ
B. taeniabronchum		Δ									Δ	ΔΔ
B. thamnobates	▲▲■							■		■ΔΔΔ		▲▲▲

A



B

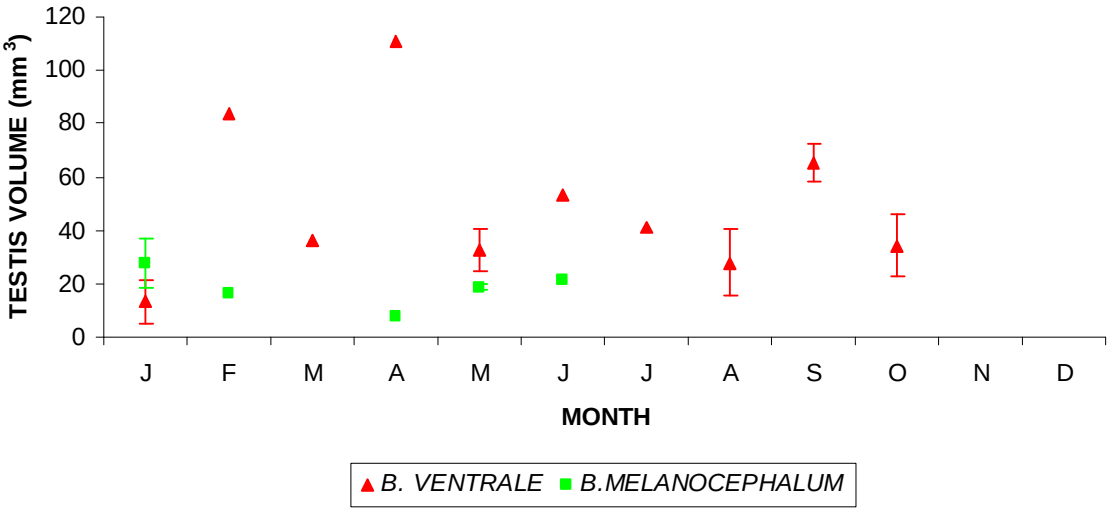


Figure 5.7 Variation of testicular volume in (A) *Bradypodion pumilum and B. transvaalense* and (B) *Bradypodion ventrale* and *B. melanocephalum.* Means and standard errors are plotted.

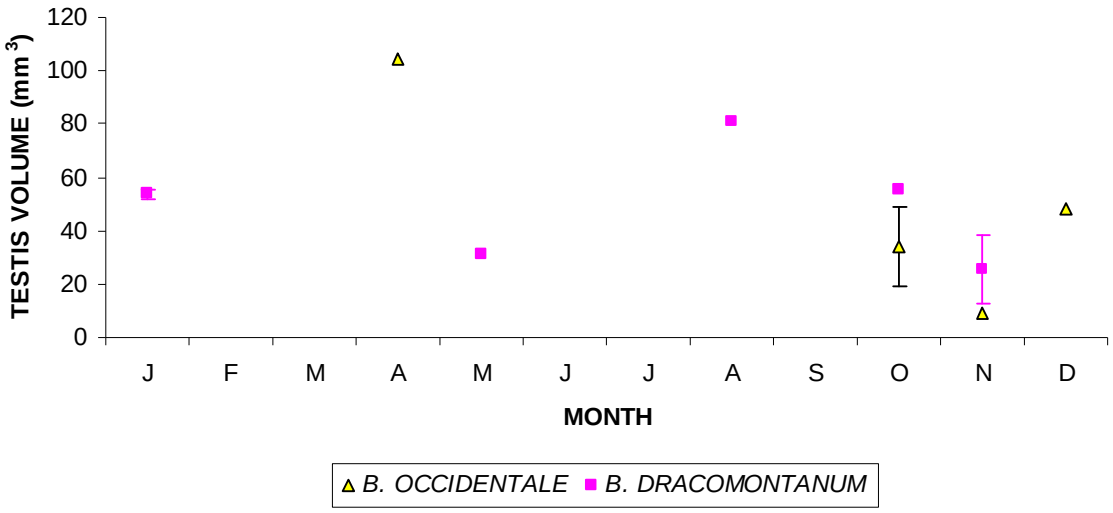
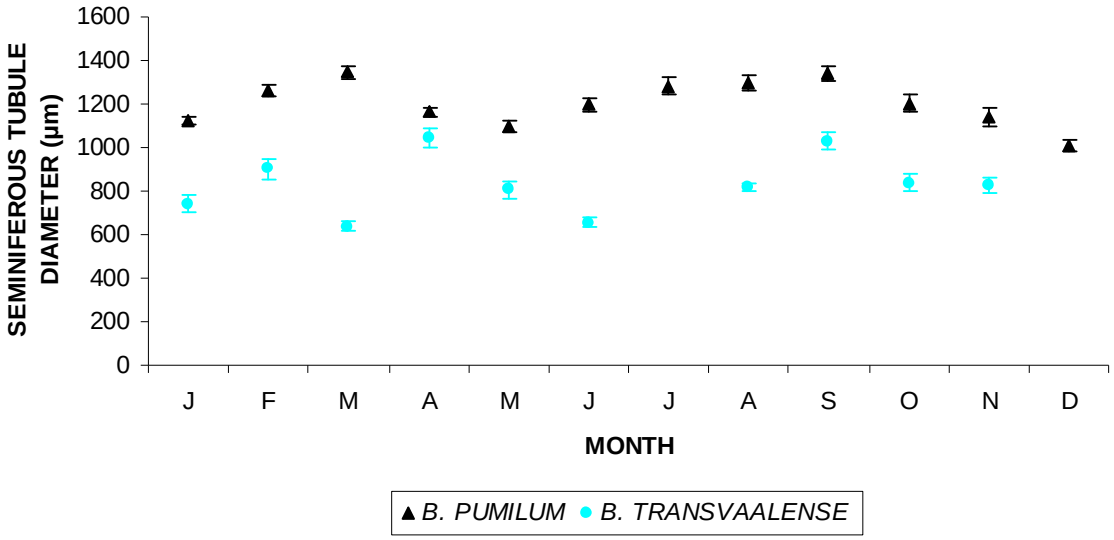


Figure 5.8 Variation of testicular volume in *Bradypodion occidentale* and *B. dracomontanum* throughout the year. Means and standard errors are plotted.

A



B

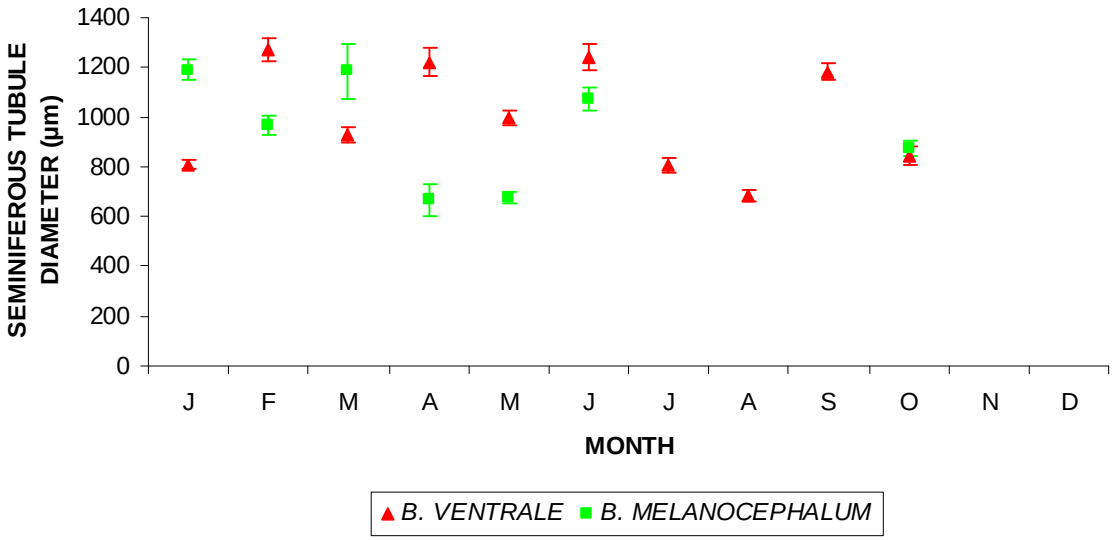


Figure 5.9 Variation of seminiferous tubule diameters in *Bradypodion pumilum, B. transvaalense* (A), *B. ventrale* and *B. melanocephalum* (B) throughout the year. Means and standard errors are plotted.

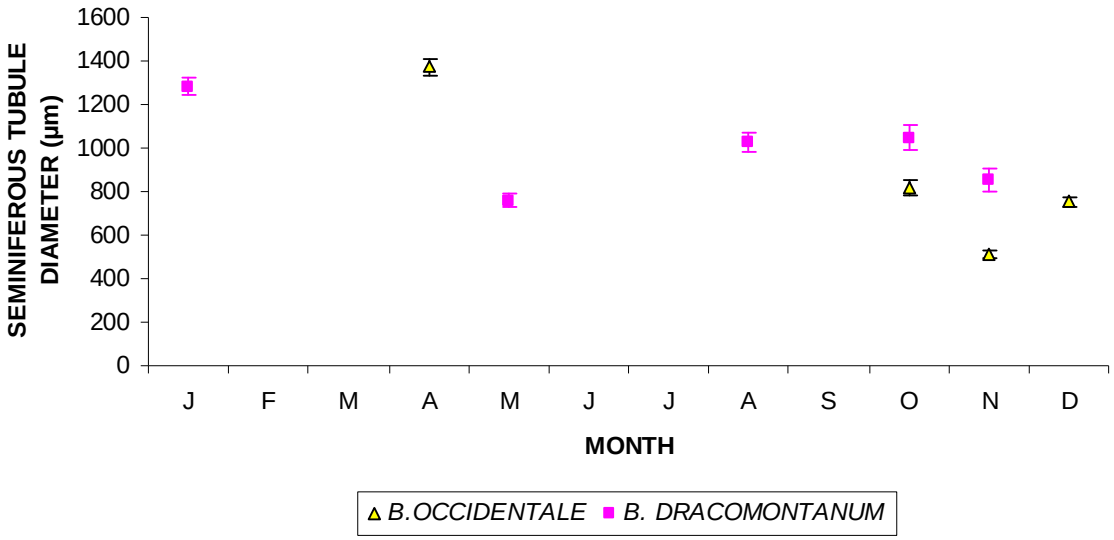


Figure 5.10 Variation of seminiferous tubule diameter in *Bradypodion occidentale* and *B. dracomontanum* throughout the year. Means and standard errors are plotted.

Table 5.3 Table showing the variation in spermatogenic activity of *B. pumilum* (A) and *B. transvaalense* (B), amongst individual males, throughout the year. Stages 1-7 defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced.

A

	SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE							
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	10			1	2	7		
F	11				1		7	3
M	11						11	
A	8					1	6	1
M	5				1		1	3
J	4		1	3				
J	3			1	1		1	
A	3			1	1		1	
S	3					1	2	
O	4						4	
N	4						4	
D	7		1	4	2			

B

	SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE							
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	3				2		1	
F	1			1				
M	3		1	1	1			
A	4			1	3			
M	1						1	
J	2			1	1			
J	0							
A	2			2				
S	1			1				
O	3					2	1	
N	2				1		1	
D	0							

Table 5.4 Table showing the variation in spermatogenic activity in *B. ventrale* (A) and *B. melanocephalum* (B) amongst individual males, throughout the year. Stages 1-7 defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced.

A

		SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE						
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	3		1	1	1			
F	1						1	
M	1				1			
A	1						1	
M	3					1	2	
J	1						1	
J	1		1					
A	2						1	1
S	3			1		1		1
O	3					2		1
N	0							
D	0							

B

		SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE						
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	3					1	2	
F	1					1		
M	1					1		
A	1						1	
M	2						2	
J	1					1		
J	0							
A	0							
S	0							
O	3			1			2	
N	0							
D	0							

Table 5.5 Table showing the variation in spermatogenic activity in *B. occidentale* (A) and *B. dracomontanum* (B) amongst individual males, throughout the year. Stages 1-7 defined as: 1, seminiferous tubules involuted with only spermatogonia; 2, primary spermatocytes appearing; 3, secondary spermatocytes and early spermatids abundant; 4, transforming spermatids with a few spermatozoa; 5, spermatids and spermatozoa abundant; 6, spermatozoa abundant (maximum level of spermiogenesis); 7, spermatozoa abundant but spermatids and spermatocytes are greatly reduced.

A

		SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE						
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	0							
F	0							
M	0							
A	1					1		
M	0							
J	0							
J	0							
A	0							
S	0							
O	2				1			1
N	1				1			
D	1			1				

B

		SPERMATOGENIC STAGE WITH NUMBER OF SPECIMENS IN EACH STAGE						
MONTH	***N***	**1**	**2**	**3**	**4**	**5**	**6**	**7**
J	3					1	2	
F	0							
M	0							
A	0							
M	1					1		
J	0							
J	0							
A	1			1				
S	0							
O	1			1				
N	2			2				
D	0							

DISCUSSION

Female dwarf chameleons

Using *B. pumilum*, *B. transvaalense*, *B. ventrale*, *B. occidentale* and *B. melanocephalum* females as representatives of the entire genus, an aseasonal reproductive cycle with a relatively high clutch size for their body size are observed. Only *B. transvaalense* showed clutch size variation throughout the year with low clutch sizes in February but this could be an artefact of the small sample size (only one gravid female was available for February). The occurrence and high percentage of gravid females present throughout the year also suggests that individual females produce multiple clutches per year. Multiple clutching may be a direct cause of the observed aseasonal reproduction in *Bradypodion*. In a viviparous species, such as *Bradypodion*, it is practically impossible to be multi-clutched and seasonal due to the time constraints of carrying a clutch.

Male dwarf chameleons

Although male sample sizes are low in most species of dwarf chameleons investigated, some general conclusions can be made from the data. It appears that *Bradypodion* males show an extended spermatogenic cycle, possibly bimodal (most pronounced in *B. pumilum*) but sperm appears available throughout the year. More samples are needed of all species to specifically determine the spermatogenic cycle of *Bradypodion*.

Dwarf chameleon reproduction

The species considered in this study represent all of the well supported clades in the *Bradypodion* phylogeny, are found in a wide range of habitat types and climatic

regimes but show similar reproductive cycles. In temperate environments, some resources are seasonally limited and are known to have considerable effects on reptilian reproductive cycles (Licht 1984; Dunham *et al*. 1988; Diaz-Paniagua *et al*. 2002). However, dwarf chameleons regardless of habitat and climatic conditions are able to reproduce throughout the year. It has also been demonstrated that both sexes of *B. pumilum* are able to store sperm (Chapters 3 and 4) and it is expected that this character would be present in all *Bradypodion* species. This type of reproductive strategy is not only highly unusual for viviparous, temperate-zone lizards (Duvall *et al.* 1982; Licht 1984; James and Shine 1985) but it is also an unusual reproductive strategy for southern African lizards (Table 1.1).

It has been suggested that the unusual reproductive strategy in *B. pumilum* may be an adaptation to inhabiting a fire-driven environment (Chapters 2, 3 and 4). *Chamaesaura anguina*, a viviparous grass lizard from the Western Cape, South Africa, also shows an aseasonal female reproductive cycle and high clutch sizes and a post nuptial male cycle which is highly unusual for the Cordylidae (Du Toit *et al.* 2003). With both *B. pumilum* and *C. anguina* inhabiting pyrophillic vegetation it was originally thought that the unusual reproduction observed in these species was due to inhabiting an unpredictable environment. This now appears an unlikely explanation in *Bradypodion* as although several species inhabit fire-prone vegetation other members of the *Bradypodion* genus show the same female reproductive characteristics but are found in a variety of different vegetation types, many of which are not fire-driven. It is possible that the ancestral *Bradypodion* adapted to an unpredictable fire-prone habitat by aseasonally reproducing. However, the *Bradypodion* date of origin (9.7 – 18.5 million years ago; Tolley *et al.*

2006) precedes the date of origin of the fire-prone fynbos vegetation approximately 2 – 5 million years ago (Cowling and Richardson 1995). Fire as the cause for the unusual reproductive characteristics observed in *Bradypodion* now appears an unlikely explanation. Nevertheless, the unusual reproductive strategy appears to be beneficial in this type of environment, not only for *Bradypodion* species inhabiting these unpredictable areas but for other species too (Catling and Newsome 1981; Du Toit *et al.* 2003). It is possible that the atypical reproductive strategy of *Bradypodion* may have assisted the original dispersal and subsequent survival of species in the fire-prone vegetation. This statement is further supported by the 'Cape Clade' of *Bradypodion* identified in Tolley *et al.* (2006) which coincides with the origin of the fire-prone vegetation.

Chameleon reproduction

Detailed scientific studies of reproduction in other species of chameleon are few and limited to the genus *Chamaeleo*. *Bradypodion* is basal to *Chamaeleo* in some chameleon phylogenies thus making an ancestral reproductive state impossible to deduce from the literature (Raxworthy *et al.* 2002; Towsend and Larson 2002). *Chamaeleo chamaeleon* is an oviparous species that inhabits a seasonal Mediterranean environment similar to *Bradypodion* but displays a seasonal female cycle (Diaz-Paniagua *et al.* 2002). Another study by Lin & Nelson (1981) studied two sympatric species from the Kenyan highlands, *C. jacksonii* and *C. hoehnelii*. Both species have different reproductive cycles despite inhabiting the same area with *C. jacksonii* showing a seasonal reproductive cycle whilst *C. hoehnelii* reproduces continuously throughout the year. The authors suggest that this is due to a higher predation pressure on *C. hoehnelii.* It is generally thought that

Bradypodion (both adults and juveniles) are particularly vulnerable to predators such as spiders, snakes, birds and domestic cats (Branch 1998; Tolley and Burger 2007), a high reproductive output could counteract this high mortality. *Chamaeleo hoehnelli* like *Bradypodion* reproduces throughout the year; however, unlike *C. hoehnelli*, *Bradypodion* inhabit temperate environments whilst *C. hoehnelli* inhabits tropical environments. It is possible that this high predation pressure on *Bradypodion* may take precedence over the pressure for *Bradypodion* to seasonally reproduce in its temperate environment. High predation makes it essential to produce many offspring many times a year and this may have resulted in aseasonal reproduction in *Bradypodion*. As viviparous species, the time constraints on producing a clutch make it virtually impossible to produce several clutches within a season. After females mature they may start to produce clutches immediately. Continual reproduction from this first clutch and subsequent addition of newly mature females, to the reproductive population, consequently cause the aseasonal reproductive cycle of *Bradypodion*.

CONCLUSION

Bradypodion show unusual reproductive characteristics; aseasonality and high fecundity in a viviparous lizard that inhabit temperate environments. It appears that this reproductive strategy may be a pre-adaptation to past environmental conditions or may be associated with a high predation pressure. Exploring past environmental conditions around the *Bradypodion* date of origin may help to uncover possible explanations for their extraordinary reproductive strategy.

CHAPTER SIX

THE ORIGIN OF VIVIPARITY AND EXTRAORDINARY REPRODUCTION IN DWARF CHAMELEONS

INTRODUCTION

It is generally accepted that squamate viviparity arose from oviparity by females retaining eggs in cold climates, to provide an enhanced thermal environment for developing embryos (Shine 1985a; 1995). In addition to parity mode, squamate reproduction may be affected by many factors. Generally, two basic kinds of reproductive cycles are known, aseasonal and seasonal (Licht 1984). Temperate-zone reptiles are usually seasonal reproducers but aseasonal reproduction, although unusual, has been ascertained (Du Toit *et al*. 2003). Tropical reptiles may show either seasonal or aseasonal breeding, subject to resource availability and environmental conditions (Licht 1984). Life history strategies are varied among squamates, even within a parity mode. Although most viviparous lizards exhibit delayed reproduction and one large clutch per season, early maturing species with multiple clutches per season are not uncommon (Dunham *et al.* 1988)

Chameleon reproductive cycles have received little attention in the literature and previous studies have focused on factors specifically affecting the female reproductive cycle (Menzies 1958; Cuadrado and Loman 1999; Diaz-Paniagua *et al*. 2002). Of approximately 150 - 160 species of chameleons (Tolley and Burger 2007), reproductive data are available for only five species, excluding this study on *Bradypodion* (Table 7.1). The reproductive studies completed show that chameleon reproduction may be varied. Species may be oviparous or viviparous, seasonal or aseasonal, and multi-clutched or single clutched but clutch size appears consistently high (Table 7.1).

Dwarf chameleons (*Bradypodion*) of southern Africa do not conform to the general trends of squamate reproduction outlined above. *Bradypodion* are temperate-zone viviparous lizards that aseasonally reproduce, with extremely high fecundity, regardless of environmental conditions (Chapters 2, 3 and 5). This type of reproductive strategy is highly unusual for viviparous, temperate-zone lizards (Duvall *et al.* 1982; Licht 1984; James and Shine 1985). Therefore, the aim of this chapter was to uncover possible drivers for the evolution of this extraordinary reproductive strategy in *Bradypodion*, with discussions in relation to other chameleons, the sister taxa of chameleons, and in relation to the biogeographic history of southern Africa.

Bradypodion originated in the Miocene approximately 14.1 ± 4.4 million years ago in south-western South Africa (Townsend and Larson 2002; Tolley *et al.* 2006). A south polar ice cap formed between 13 – 16.5 million years ago and the Benguela current and upwelling system began to develop along the west coast of southern Africa (Siesser 1978; 1980; Woodruff *et al.* 1981). This caused the climate to become cool and dry in south-west Africa. All species basal to *Bradypodion* in the chameleon phylogeny are oviparous (Raxworthy *et al.* 2002; Townsend and Larson 2002; Matthee *et al*. 2004) thus this cooling of the climate may have started the transition from oviparity to viviparity in *Bradypodion.*

Interestingly, a group of melanistic lizard species have their origin in the south-western region of South Africa at this time (Daniels *et al.* 2004). Daniels *et al.* (2004) state that melanism evolved between 17 and 15 million years ago in these lizards due to a single climatic event. Presently these lizards are found in areas

associated with cooler climates due to limited solar radiation as a result of frequent fog and cloud cover and it is suggested that conditions were similar during the evolution of these cold-adapted forms (Daniels *et al.* 2004). It is thought that these melanistic lizards are relicts from what were once much larger populations. This further supports the effect of the cool period on lizard species in this region and hence the transition from oviparity to viviparity in *Bradypodion* at this time.

FAMILY CONNECTIONS

Bradypodion is part of the family Chamaeleonidae, a member of the order Iguania, which also contains the families Agamidae and Iguanidae. Both agamids and iguanids are widespread throughout the world. The Agamidae is considered the old world equivalent of the new world Iguanidae (Honda *et al*. 2000). Chamaeleonidae occur in Africa, Madagascar, the Middle East, India and Sri Lanka, and southern Spain in a wide variety of habitats and climate regimes (Pough *et al.* 2001; Tolley and Burger 2007). It is known that they occur in other southern European countries but these are thought to be introduced populations (Pough *et al.* 2001). The family Chamaeleonidae contains nine genera; *Brookesia, Rieppeleon, Rhampholeon*, *Bradypodion*, *Kinyongia*, *Nadzikambia*, *Calumma*, *Chamaeleo* and *Furcifer* (Matthee *et al.* 2004; Tilbury *et al.* 2006; Tolley and Burger 2007).

Origins of viviparity

Iguanians are predominantly oviparous, although multiple origins of viviparity are evident. Approximately 12 are within the Iguanidae, two are within the Agamidae and two are within the Chamaeleonidae (Blackburn 1999). The two viviparous

lineages of chameleons are the southern African *Bradypodion* and the East African *C. bitaeniatus* group (Blackburn 1999).

Conflicting phylogenies in the literature make it difficult to comment on possible drivers for the evolution of viviparity in the East African viviparous *Chamaeleo bitaeniatus* group at the time of divergence (Raxworthy *et al.* 2002; Towsend and Larson 2002; Matthee *et al*. 2004; Tilbury *et al.* 2006). Raxworthy *et al.* (2002) date the divergence between the *Chamaeleo* and *Furcifer* genera at approximately 28 – 58 million years ago. However, Matthee *et al.* (2004) state all other chameleon genera diverged from the basal *Brookesia* around 30.5 – 34.86 million years ago (this split is defined as 35 – 68 million years ago in Raxworthy *et al.* (2002)). Presently the East African viviparous lineage inhabits the tropical East African highlands (Pilley 2000) and climatic and topographic changes took place in East Africa during the late Paleogene and early Neogene. On the boundary of the Eocene and Oligocene the Earth's climate drastically changed from what is dramatically termed a 'greenhouse to an icehouse' (Elderfield 2000). Although summer temperatures are thought to have remained the same, winter temperatures dropped by up to 4 ºC (Elderfield 2000; Ivany *et al.* 2000). Also around this time (approximately 30 million years ago) the coastal lowlands of Eastern Africa were probably submerged intermittently (Burgess *et al.* 1998; Zachos *et al.* 2001) possibly confining species to higher areas. In addition to global climatic shifts (and local sea level changes) the uplifting of the East African Rift would also have had a profound effect on the local biota. The eastern section of the East African Rift system started to uplift during the Eocene and Oligocene reaching a maximum in the Plio-Pleistocene with most of the activity occurring

approximately 10 – 25 million years ago (Partridge 1995; Sepulchre *et al.* 2000). The western section of the East African Rift system developed during the Miocene (Sepulchre *et al.* 2000). These tectonic changes also triggered the aridification of East Africa (Sepulchre *et al*. 2000). Due to these environmental changes it is possible that the confinement to higher altitudes, the general uplifting of East Africa and the global drop in temperatures could cause a shift from oviparity to viviparity in the *C. bitaeniatus* group.

Generally, it seems that the two viviparous lineages in Chamaeleonidae presently occur in cold and arid environments (*Bradypodion*) or tropical environments at high elevations (*C. bitaeniatus* group). This appears true for viviparous lineages in both the Agamidae and Iguanidae. Of the two transitions to viviparity in Agamidae one occurs in cold arid areas (*Phrynocephalus* group) whilst the other occurs in tropical montane cloud forests (*Cophotis ceylanica*) (Pang *et al.* 2003; Bahir and Surasinghe 2005). Little is known about the reproductive strategy of *Cophotis ceylanica*. Although bearing an uncanny resemblance to *Bradypodion* (small, arboreal, laterally compressed agama with a pre-hensile tail), it occurs in the moist tropical montane forests of Sri Lanka (Bahir and Surasinghe 2005). It is possibly in this environment that viviparity evolved in this species.

The origin of viviparity in *Phrynocephalus* has received some attention in the literature. Certain members of the genus are viviparous whilst others remain oviparous. *Phrynocephalus* are wide-spread, sand lizards occurring from China to Turkey (Pang *et al.* 2003). A study by Pang *et al.* (2003) illustrates the transition to viviparity in the Chinese *Phrynocephalus* species. Of the 18 Chinese species six

are viviparous. These viviparous species are endemic to the region and mostly restricted to the cold, arid, highland areas (Qinghai-Xizang (Tibetan) plateau) whilst lowland populations are oviparous (Pang *et al.* 2003). During the Miocene the area became arid due to rain shadowing by the uplifting Himalayan Mountains and subsequent uplifting in the Pliocene caused the climate to cool (Pang *et al.* 2003). Pang *et al*. (2003) suggest that the divergence between the oviparous and viviparous clades occurred due to this cooling and aridifcation of the area.

Similar transitions to viviparity also occur within Iguanidae with species occurring in cold-arid areas, and tropical-montane areas. The viviparous Phrynosomatidae (including *Phrynosoma* and *Sceloporus (formosus, grammicus, torquatus, angustus, goldmani and bicanthalis* groups)) and the viviparous Tropiduridae (including *Ctenoblepharis nigriceps*, *Liolaemus*, *Phymaturus* and *Vilcunia*) are all associated with cold arid areas and high altitudes (Guillette *et al.* 1980; Schulte *et al.* 2000; Hodges 2004). In contrast, *Corytophanes pericarinatus* is from a tropical environment.

In conclusion, the viviparous Iguania species appear to occur in two distinct categories. The first category is species inhabiting a cold and arid environment and the second, species inhabiting a tropical, montane environment. This however, is based mainly on current distributions rather than conditions under which viviparity originated. *Bradypodion* fall in to the cold-arid grouping.

What causes unusual reproduction?

It was originally thought that *B. pumilum* and possibly the ancestral *Bradypodion* may have been reproductively adapted to life in the pyrophillic fynbos by aseasonally producing large clutches, several times a year (Chapter 2, 3 and 4). However, both the *B. pumilum* (5 – 12.2 million years ago) and the *Bradypodion* date of origin (9.7 – 18.5 million years ago) precedes the date of origin of the fire-prone fynbos vegetation approximately 2 – 5 million years ago (Cowling and Richardson 1995; Tolley *et al.* 2006). Although there are records of fires before the origin of the fynbos it was thought that the increase in the incidence of fires after this would be the driver for the unusual reproductive strategy seen in the *Bradypodion*. The hypothesis that the ancestral *Bradypodion* exhibits unusual reproductive characteristics due to the fynbos fires now appears an unlikely explanation. Nevertheless, the unusual reproductive strategy of *Bradypodion* appears to be beneficial in this type of environment, not only for the *Bradypodion* species inhabiting these unpredictable areas but for other species too (Catling and Newsome 1981; Du Toit *et al.* 2003). It is possible that the atypical reproductive strategy of *Bradypodion* may have assisted the original dispersal and subsequent survival of populations in fire-prone vegetation. This statement is further supported by the 'Cape Clade' of *Bradypodion* identified in Tolley *et al.* (2006) which coincides with the origin of the fire-prone vegetation.

An alternate explanation of aseasonal reproduction in *Bradypodion* is that during the Oligo-Miocene (when *Bradypodion* originated), southern African vegetation was dominated by sub-tropical forests (Axelrod and Raven 1978). It is likely that the reproductive aseasonality observed in *Bradypodion* is a retained trait from this

type of environment. Since the Miocene the sub-tropical forests of southern Africa have withdrawn considerably thus leaving *Bradypodion* aseasonally reproducing in its now Mediterranean environment.

Reproductive aseasonality within the Chamaeleonidae is not unique to *Bradypodion*. Viviparous, *Chamaeleo hoenelii* from tropical East Africa also reproduce aseasonally. An ancestral trait of aseasonal reproduction can not be concluded for *Bradypodion* as the position of *Chamaeleo* (in relation to *Bradypodion*) in the chameleon phylogeny is currently unresolved. Nonetheless, Raxworthy *et al.* (2002) suggest that all chameleons originated in tropical Madagascar. In addition, *Brookesia* and to some extent *Rhampholeon* are said to be basal lineages in the chameleon phylogeny (Raxworthy *et al.* 2002; Towsend and Larson 2002; Matthee *et al*. 2004; Tilbury *et al.* 2006). *Brookesia* are from tropical Madagascar whilst *Rhampholoen* are from tropical Africa. *Bradypodion* may be retaining the ancestral state of aseasonal reproduction but the reproductive characteristic of both *Brookesia* and *Rhampholeon* are relatively unknown. Thus again demonstrating the tropical affinities of *Bradypodion*.

A third scenario is that climatic fluctuations may have caused the reproductive aseasonality in *Bradypodion*. During the Quaternary the environment on the south-western side of Africa was apparently particularly variable (Deacon and Lancaster 1988). For example, the environment changed from warm temperatures to cool temperatures, from arid to moist conditions, from windy to still conditions and the seasonality of rainfall fluctuated from summer to a more Mediterranean rainfall

pattern on many occasions. It is thought that this variable habitat may have played a role in the aseasonal cycle now observed in *Bradypodion*.

Despite past conditions aseasonality persists in *Bradypodion*. It is thought that due to the relatively mild present climate of South Africa, neonate *Bradypodion* have an equal chance of survival in all seasons of the year. In addition, mortality in dwarf chameleons is thought to be particularly high year round regardless of the season.

High reproductive output

Bradypodion have an extremely high reproductive output for small, viviparous lizards. Generally viviparous lizard species appear more K-selected however *Bradypodion* is r-selected. It is thought that their arboreal lifestyle and small size makes them particularly vulnerable to predation. The aforementioned *Chamaeleo hoehnelii*, from the tropical highlands of Kenya, is a small, viviparous, early-maturing, aseasonal breeder, producing two clutches of small young per year (Lin and Nelson 1981). A high predation rate (like in *Bradypodion*) is suggested for the high reproductive output in *C. hoehnelii*.

A high reproductive output may be advantageous in a fire-prone environment. *Bradypodion pumilum* (as a representative of all *Bradypodion* species in a fire-prone area) inhabit fire-driven vegetation. The occurrence of fire in the environment of *B. pumilum* would have the same ultimate affect as a predator; risk of death or injury. Populations may be decimated after a fire and high fecundity may help to ensure a rapid recovery of the population before the next fire.

SOUTHERN AFRICAN CONNECTION

In addition to *Bradypodion*, southern Africa has other lizards showing unusual reproductive strategies for temperate-zone lizards. The Southern Rock Agama, *Agama atra* is endemic to southern Africa occurring on rocky outcrops throughout the region (Branch 1998). Agamas are oviparous, seasonal breeders (even within the tropics) with the exception of the Namaqualand population of *Agama atra atra* (Fitch 1970; Van Wyk 1983; James and Shine 1985; Heideman 1994; Mouton and Herselman 1994). The *Agama atra atra* population from Namaqualand (hereafter referred to as Namaqua *Agama a. atra*) reproduces aseasonally and its reproductive strategy may be considered bet-hedging as they are relatively large, late maturing animals (Mouton and Herselman 1994). Thus as previously discussed, aseasonal reproductive cycles are generally associated with either a tropical or unpredictable environment. Namaqualand is neither a tropical or unpredictable environment with relatively mild desert conditions, highly predictable annual winter rainfall and moderate temperatures (Cowling *et al*. 1999). With a predictable, seasonal environment such as this, the observation of an aseasonal reproductive cycle in Namaqua *Agama a. atra* is therefore unusual. Mouton and Herselman (1994) suggest that Namaqua *Agama a. atra* is a 'tropical leftover' thereby retaining reproductive aseasonality in the now seasonal environment of Namaqualand. Furthermore, the authors noted two typically tropical lineages occurring in the area. One is the Namaqua Day Gecko, *Phelsuma occelata* and the other the Cape Flat Lizard, *Platysaurus capensis.* No detailed reproductive studies exist for *Phelsuma occelata* however anecdotal observations suggest that reproduction may be seasonal (Branch 1998). In addition, both male and female *Platysaurus capensis* show seasonal reproductive cycles (Broadley 1974; Van

Wyk and Mouton 1996). If all three species are 'tropical leftovers' why is Namaqua *Agama A. atra* the only species to reproduce aseasonally and in a very predictable seasonal environment? Matthee and Flemming (2002) suggest that divergence within the clades of *Agama a. atra* took place approximately 2.2 – 4.4 million years ago due to climatic fluctuations. The west coast of Africa was a particularly harsh environment during this time due to climatic fluctuations (Deacon and Lancaster 1988) and it is possible that during these changeable conditions Namaqua *Agama a.* atra (referred to as the northern-central clade in Matthee and Flemming (2002)) became aseasonal reproducers. In conclusion, it appears that climatic fluctuations of the Pliocene may have caused reproductive aseasonality in Namaqua *Agama a. atra.* Interestingly, *Chamaeleo namaquensis* (oviparous, ground living and large body size) also occurs in this area and shows a seasonal but prolonged reproductive cycle (Burrage 1973).

The second species to show an unusual reproductive strategy is The Cape Grass Lizard, *Chamaesaura anguina*. The southern African cordylids are conservative seasonal reproducers with relatively low fecundity (Van Wyk 1990; Van Wyk and Mouton 1991; Flemming and Van Wyk 1992; Flemming 1993a; Van Wyk and Mouton 1995; Van Wyk and Mouton 1996; Flemming and Mouton 2002). Reproductive activity in female cordylids is confined to autumn and winter. Male cordylids may show either a pre-nuptial or post-nuptial spermatogenic cycle but are always seasonal (Van Wyk 1990; Van Wyk and Mouton 1991; Flemming and Van Wyk 1992; Flemming 1993a; Van Wyk and Mouton 1995; Van Wyk and Mouton 1996; Flemming and Mouton 2002). Conversely, the Cape Grass Lizard, *C. anguina* exhibits a curious reproductive cycle. Whilst all Cordylidae (with the

exception of *Platysaurus*) are viviparous, *C. anguina* is a viviparous, aseasonally reproducing lizard with extremely high fecundity (Du Toit *et al.* 2003). In addition, female *C. anguina* attain larger body sizes than males to allow for much larger clutch sizes (Du Toit *et al.* 2003). In fact, the degree of sexual size dimorphism observed in *C. anguina* is also highly unusual for cordylids (Du Toit *et al.* 2003).

It is believed that viviparity evolved in cordylids in south-western Africa in the immediate ancestor to the *Cordylus* clade (*Chamaesaura*, *Cordylus* and *Pseudocordylus*) due to cold climates (Mouton and Van Wyk 1997). The formation of the Benguela current cooled the climate and caused aridification of south-western Africa. As a result, the rainforests present in south-western Africa began to withdraw and were replaced by sclerophyllous plants (Cowling and Richardson 1995). Due to this combustible vegetation, fires became more frequent and approximately 5 - 2 million years ago the fynbos was established (Cowling and Richardson 1995).

It is thought cool climates (possibly the formation of the Benguela current), as in *Bradypodion*, may be responsible for the evolution of viviparity in cordylids of this region. In support of this the Cordylidae (*Chamaesaura*, *Pseudocordylus*, *Cordylus* and *Platysaurus*) with the exception of *Platysaurus* are associated with temperate, montane habitats (Mouton and Van Wyk 1997). *Platysaurus* (the only oviparous genus in Cordylidae) is more associated with lowland and subtropical areas.

In conclusion, the formation of the Benguela current upwelling system caused the climate to become cool and arid in south-western Africa. It is possible that this

change in environment initiated the transition from oviparity to viviparity in the cordylids of south-western Africa. With this transition to viviparity seems to have come a K-selected, seasonal strategy with all the other cordylids. However, *C. anguina* may not have been able to retain this K-strategy in an arboreal environment. It is also thought that due to the increase in the incidence of fires in the Western Cape due to the proliferation of more sclerophyllous plants a K-selected seasonal cycle became redundant. To survive in this type of unpredictable environment *C. anguina* required an aseasonal reproductive cycle with high clutches to repopulate the areas rapidly after a fire.

CONCLUSION

Viviparity within the Iguania appears to fall into two distinct groupings; cold and arid environments and tropical, high elevations. *Bradypodion* seems to fall into the cold-arid group. It is likely that the cooling of the climate due to the development of the Benguela current facilitated the transition to viviparity in *Bradypodion*. *Bradypodion* may be aseasonal reproducers for a number of reasons. They are of tropical ancestry, they relatively recently inhabited tropical forests, or fluctuations in climate may have caused this. *Bradypodion* most likely have a high reproductive output due to their intense vulnerability to predation as in other chameleon species.

The proposed hypothesis that the unusual reproductive characteristics of *B. pumilum* (and possibly the ancestral *Bradypodion*) were due to inhabiting a fire-prone environment now appears an unlikely explanation. However, even if this

extraordinary reproduction was not in direct response fire, the strategy appears beneficial in this type of unpredictable environment.

Three taxa in southern Africa exhibit similar reproduction but appear to have different life history evolutions. As a result of the development of the Benguela upwelling system, the cooling of the climate caused the transition from oviparity to viviparity, in both *Bradypodion* and possibly the viviparous Cordylidae. Subsequent to this Namaqua *Agama a. atra* and *Bradypodion* either became aseasonal because of past environmental conditions or retained previous aseasonality from tropical ancestry. Conversely, *C. anguina* appears to have developed aseasonality specifically for life in a fire-driven environment. The high reproductive output of *B. pumilum* and *C. anguina* may help to counteract high mortality caused by fire or predation.

Table 7.1 Reproductive characteristics of chameleons. CS = clutch size, C/Yr = clutches per year. Asterisks indicate mean value.

SPECIES	REPRODUCTIVE MODE	REPRODUCTIVE CYCLE	CS	C/Yr	CLIMATE	REFERENCE
C. gracili	Oviparous	Seasonal	45	1	Seasonal tropics	Menzies 1958
C. namaquensis	Oviparous	Seasonal	13.2*	2 – 3	Seasonal	Burrage 1973
C. chamaeleon	Oviparous	Seasonal	4 – 40	1	Seasonal Mediterranean	Diaz-Paniagua *et al*. 2002; Cuadrado and Loman 1999
C. hoehnelii	Viviparous	Aseasonal	13.7*	2	Aseasonal tropics	Lin and Nelson 1981
C. jacksonii	Viviparous	Seasonal	22.3*	1	Aseasonal tropics	Lin and Nelson 1981

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